Bacterial Adherence of *Staphylococcus Epidermidis* to Intraocular Lenses: A Bioluminescence and Scanning Electron Microscopy Study

Laurent Kodjikian,1,2,3 Carole Burillon,2,3 Christine Roques,4 Gérard Pellon,5 Jean Freney,3,6 and François N. R. Renaud5,6

**PURPOSE.** To analyze and compare the adherence of *Staphylococcus epidermidis* to intracocular lenses (IOLs) made of five different biomaterials (native or heparinized polymethylmethacrylate, silicone, hydrophilic acrylic, or hydrogel) and to detail the different steps and mechanisms of bacterial adhesion to a polymer.

**METHODS.** A clinical strain carrying the intercellular adhesion (ica) locus was used. In a previous study, the extent of bacterial binding was measured by counting. In this study, two different techniques, bioluminescence and scanning electron microscopy (SEM), were used to analyze the accuracy of each one, to obtain a comparison between the various IOLs, and to complete previous observations. The results were compared using both the Kruskal-Wallis and the Mann-Whitney nonparametric tests.

**RESULTS.** Bacterial adhesion was statistically weakest on hydrogel and then on hydrophilic acrylic polymer. Adhesion depended on the hydrophobicity or hydrophilicity of the biomaterials. Slight differences were found between the two methods, and these differences are explained. Furthermore, SEM observations highlighted two different patterns of bacterial adhesion (isolated bacteria and clusters of bacteria), assuming that hydrophobic IOLs (silicone and PMMA) probably facilitate bacterial colonization and biofilm production.

**CONCLUSIONS.** Attachment mechanisms may be different in each case, depending on the polymer material and the infecting organism, because there are various types of behavior among *S. epidermidis* strains. Hydrophilic polymer surfaces (hydrogel and probably hydrophilic acrylic) seem to be useful in avoiding the development of bacterial colonies and hence in preventing endophthalmitis. Fewer bacteria were attached, demonstrating inhibition or delay in bacterial colonization. *(Invest Ophthalmol Vis Sci. 2003;44:4388–4394) DOI:10.1167/iovs.03-0186*

Endophthalmitis is still one of the most serious complications after cataract surgery with intraocular lens (IOL) implantation. The disease remains difficult to anticipate and to diagnose and represents a therapeutic emergency because of its rapid evolution and poor prognosis.

It is well known that the binding of bacteria on IOLs during implantation represents a prominent factor in the pathogenesis of postoperative endophthalmitis and of pseudophakic chronic intraocular inflammation.2–6 One may hope to decrease the occurrence of postoperative endophthalmitis by reducing bacterial adherence to intraocular implants.6,7 *Staphylococcus epidermidis* is the most common pathogen causing acute postoperative endophthalmitis.8,9 We have studied the adherence of *S. epidermidis* (clinical strain N890074) to IOLs made of different, more or less hydrophilic, biomaterials.8 The extent of lens bacterial contamination was measured by releasing bound bacteria by sonication in buffers; the resultant suspension was spread on nutritive agar plates, and cultured colonies were counted. Adherence was weakest on hydrogel and strongest on the silicone polymer, but there were no significant results for native or heparinized polymethylmethacrylates (PMMAs) and hydrophilic acrylics. Bacterial adherence to the implant surface thus seemed to depend on the hydrophobicity or hydrophilicity of the biomaterial.

Nevertheless, even if bacterial counting seems to be the classic reference method, it may not have a very high diagnosis sensitivity or predictability. That is why it is worth looking for more reliable techniques to collect and measure biofilms.10 Thus, because the recovery efficiency of sonication methods used to quantify microorganisms attached to surfaces has recently been questioned10 and because our previous results4 were not all significant, we decided to study bacterial adherence to IOLs using two alternative techniques: bioluminescence and scanning electron microscopy (SEM), allowing the corresponding results to be compared as well as completed. Indeed, SEM observations helped us to a better understanding of the different steps and mechanisms of bacterial adherence to biomaterials by showing differences in biofilm formation depending on the different polymers. To our knowledge, no study has been published so far concerning the comparison of bacterial adherence to five commercialized kinds of IOLs (71 lens samples in all) with two different methods (bioluminescence and SEM). The hypothesis that there are differences in colonization depending on the IOLs is also new in ophthalmology.

**MATERIALS AND METHODS**

The present study was performed on 71 IOLs made of five different plastic materials: PMMA (15 lenses), heparinized (heparin surface modified [HSM]) PMMA (11 lenses), silicone (16 lenses), hydrophilic acrylic (acrylate or methacrylate polymers: 12 lenses) and hydrogel (HEMA, hydroxy-ethyl-methacrylate, or PHEMA, poly-HEMA: 17 lenses). Various firms in France manufacture these new sterile inserts (Table 1). For each of the above-cited biomaterials, three IOLs were examined by SEM, whereas all others were examined using bioluminescence.

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Strains

The microbiology department of Edouard Herriot Hospital (Staphylococci National Reference Centre, Lyon, France) provided a clinical isolate of *S. epidermidis* (N899074). This strain was isolated from infected cerebrospinal fluid due to a complication of a ventriculoperitoneal shunt in a child with hydrocephalus. N899074 was identified by colony and microscopic morphology, by the lack of coagulase activity on rabbit plasma (bioMérieux, Marcy l’Etoile, France), by the absence of production of a clumping factor (Staphyloxide; bioMérieux) and by results on a Staph gallery (ID32; bioMérieux). This isolate is able to produce a great amount of slime. Indeed, by using polymerase chain reaction amplification,11 we checked that this strain carried the intercellular adhesion (ica) locus12 which is known to encode production of *S. epidermidis* antigens mediating adhesion to biomaterials and between the bacterial cells. The bacterial concentration was spectrophotometrically adjusted to $10^8$ colony-forming units (CFU) per milliliter in a sterile 0.08 M phosphate-buffered (pH 7.8) saline solution (PBS buffer).

Methods

Lenses were incubated in the bacterial suspension for 1 hour at 37°C, with continuous shaking, before being washed three times in a 0.08 M PBS (pH 7.8) solution to remove unbound bacteria. Bacterial binding was then measured and studied by bioluminescence and SEM.

Bioluminescence Assay

The bacterial adherence to polymers was assessed using a method derived from the one proposed by Ludwicka et al. in 1985,13 Lenses were soaked in 2.5% trichloracetic acid (400 µL) for 5 minutes to extract bacterial adenosine triphosphate (ATP) and to avoid its enzyme-mediated degradation. A control was prepared at the same time by replacing the implant with 400 µL of PBS. At the end of incubation, a 60-µL sample was transferred to a tube containing 1140 µL of tris-acetate buffer (pH 7.78), giving a 1:25 dilution. The amount of ATP was measured immediately afterward. A 160-µL sample was picked and mixed with 40 µL of reagent (ATP monitoring kit from Bio-Orbit, Turku, Finland). This reagent turns ATP into adenosine monophosphate (AMP) and concomitantly releases light, which is measured by a chemiluminometer (Leader 50; GenProbe, San Diego, CA). Light emission is proportional to ATP concentration, in direct ratio with bacterial concentration. To establish the amount of bacterial ATP present in each sample, 10 µL of a standard solution of ATP (10^-7 M) was added to the medium, and the increase in light emission was measured. The ATP quantity was calculated from the ratio $(I + I_o)/I_0$, where $I$ and $(I + I_o)$ are the intensities of light emitted by bacterial ATP alone and by bacterial ATP + added standard ATP, respectively. The area of each lens depends on its diameter, haptic shape, and thickness. IOL manufacturers provided the exact area of all studied implants, which varied from 47 to 113 mm². Counts were thus expressed as picomolar (10^-12 M) of ATP per 100 mm².

SEM Method

The IOLs were first fixed with 2% (wt/vol) glutaraldehyde in a filter-sterilized 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature for 2 hours and then rinsed 3 times for 15 minutes in a 0.15 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 was followed by a quick rinse in distilled water. Fixed lenses were dehydrated step by step in ethanol. Samples were soaked, first in ethanol-water mixtures with increasing ethanol concentrations (50%, 50%, 70%, 80%, and 95% by volume, successively), for 7 minutes each, then in pure ethanol for 5, 10, and 15 minutes, successively. They were then put into an ethanol bath that was allowed to evaporate. Dried samples were stuck on metal holders with double-sided adhesive tape and finally coated in an evaporator with gold and palladium. Observations were usually performed at 5 kV with a scanning electron microscope (model S800; Hitachi, Tokyo, Japan; microscopy performed at the Scanning Electron Microscopy Centre for Applied Biology, Campus de la Doua, Lyon, France). Sixteen “observation fields,” each of them measuring $45 \times 45 \mu m$, were chosen randomly from the optic surface of each IOL. The counts were then averaged, and the number of adhering bacteria per observation field was calculated.

Statistical Method

Results were consequently presented either as picomolar of ATP per 100 mm² for bioluminescence or as an amount of bacteria per observation field for SEM. They were compared globally using a nonparametric one-factor variance analysis test, the Kruskal-Wallis test (SPSS for Windows, ver. 10.0; SPSS Inc., Chicago, IL). Then the Mann-Whitney nonparametric test was used to compare pairs of materials. $P < 0.05$ was considered statistically significant.

Results

Both the amount of ATP per unit area (so indirectly the number of bound bacteria) for bioluminescence and the number of adhering bacteria per observation field for SEM were statistically lower on hydrogel than on the four other biomaterials (Figs. 6, Tables 2, 3). The overall Kruskal-Wallis test showed that the difference between all five groups was not statistically significant ($P = 0.229$). Then the Mann-Whitney nonparametric test was used to make pair-wise comparisons between the types of biomaterials. The probabilities are shown in Table 3. The differences between hydrogel and each of the four other materials were statistically significant ($P < 0.05$), but the differences between the four other materials were not statistically significant.

With the SEM method, the number of lens-bound bacteria on hydrogel was lower than the number of bacteria on hydrophilic acrylic, which was significantly lower than the number of bacteria attached to the three other biomaterials (Figs. 6, Tables 2, 4). The overall Kruskal-Wallis test showed that the difference between all five groups was not statistically significant ($P = 0.053$). The Mann-Whitney nonparametric test was then used to make pair-wise comparisons between the types of biomaterials. The probabilities are shown in Table 4. The differences were only statistically significant when comparing hydrogel with each of the four other materials and hydrophilic acrylic with each of the four other materials. However, the

<table>
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<th>Table 1. Characteristics of the IOLs Used</th>
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<td><strong>Material</strong></td>
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<td>PMMA</td>
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<tr>
<td>Heparinized PMMA</td>
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<tr>
<td>Silicone</td>
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<tr>
<td>Hydrophilic acrylic</td>
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<tr>
<td>Hydrogel</td>
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pair-wise comparison of PMMA, heparinized PMMA, and silicone did not show any significant differences.

Moreover, standard deviations were higher for bioluminescence than for SEM (Fig. 6). Hypotheses are set forth in the Discussion section.

Finally, SEM observations showed two different patterns of bacterial adhesion: isolated bacteria on hydrogel, hydrophilic acrylic, and heparinized PMMA IOLs (Figs. 1, 2, 5), and clusters of bacteria on PMMA and silicone IOLs (Figs. 3, 4). The mean number of clusters per observation field was 5 and 17 for PMMA and silicone, respectively. Furthermore, the mean num-

**FIGURE 1.** Isolated bacteria adhering to a hydrogel surface. Original magnification, ×2000.

**FIGURE 2.** Isolated bacteria adhering to a hydrophilic acrylic surface. Original magnification, ×2000.

**FIGURE 3.** Clusters of bacteria adhering to a PMMA surface. Original magnification, ×2000.

**FIGURE 4.** Clusters of bacteria adhering to a silicone surface. Original magnification, ×2000.
The incubation time of implants in the bacterial suspension was set to 1 hour only, because our preliminary studies based on results from Ludwicka et al.\(^{15}\) showed that the number of IOL-adherent bacteria in a PBS buffer reached the highest level once the bacteria had incubated for at least 1 hour. In the literature, most of the studies used an incubation time between 0.5 and 2 hours.\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\)\(^13\)\(^14\) However, all these articles studied bacterial adhesion rather than bacterial colonization of a polymer. Colonization probably takes a few hours or maybe even days of incubation to build a complete biofilm able to embed bacteria within the whole polymer, depending on the environment, the cellular response, and cell-support interactions.\(^16\)

To gain better understanding and control of biofilms on IOLs, researchers have to develop reliable techniques. In this study, we used two original methods to analyze bacterial adherence: bioluminescence and SEM. During preliminary in vitro studies, we checked the efficiency and the sensitivity of bioluminescence compared with bacterial counting. In 1989, Kristinsson\(^{22}\) showed a valid correlation between bioluminescence measurements and bacterial counting after ultrasonication, but bacterial counting cannot detect and count viable but noncultivable bacteria, which are always present in any biofilm.\(^{23}\)\(^24\) On the contrary, bioluminescence can detect all living bacteria whether they are cultivable or not; and SEM can detect the total number of bacteria, whether they are alive or not. Thus, it was worth looking for a comparison between techniques to find more reliable ones.\(^10\)

Apart from Hogt et al.\(^{25}\) most investigators have emphasized that hydrophobicity promotes bacterial binding on surfaces.\(^2\)\(^3\)\(^26\)\(^27\)\(^28\) The results of the present work are in keeping with the literature and with our previous work using bacterial counting.\(^4\) Both studies produced the same results, except with heparinized PMMA. Silicone and untreated PMMA, the most hydrophobic polymers, yielded the highest number of adherent bacteria in a statistically significant way, in comparison to hydrogel and hydrophilic acrylic, the most hydrophilic polymers. Dankert et al.\(^{29}\) showed that a hydrophobic bacterium adhere to a hydrophobic surface more easily than its hydrophilic counterpart, and that both hydrophobic and hydrophilic bacteria adhere less easily to a hydrophilic surface.\(^{29}\) Moreover, the more hydrophilic the surface is, the less the bacteria adhere to it.\(^15\)\(^22\) Accordingly, we found that hydrogel used in the study, carried significantly fewer bound bacteria.

The number of bacteria per cluster was 40.3 (range, 6–130) and 12.5 (range, 3–60) for PMMA and silicone, respectively.

**DISCUSSION**

Coagulase-negative staphylococci are currently recognized as major etiological agents in postsurgery endophthalmitis.\(^15\) Because the treatment of this serious disease can be disappointing, preventing bacterial adherence—the first step in bacterial colonization—would be a much better approach to reducing the incidence of this complication.

**FIGURE 5.** Isolated bacteria adhering to a heparinized PMMA surface. Original magnification, ×2000.

**FIGURE 6.** Bacterial adhesion as measured by bioluminescence and SEM.
than any other tested polymer, in keeping with Ng et al. Moreover, with the SEM method, the extent of bacterial binding to hydrophilic acrylic was significantly higher than binding to hydrogel and also significantly lower than binding to silicone or PMMA.

Factors relating to the bacterium could also affect adherence, as far as heparinized implants are concerned. For instance, Tetz et al. found that an S. epidermidis strain adheres less to heparinized than to untreated PMMA, but they failed to extend this result to another strain. The same result was reported by Schmidt et al. and Schloricke et al. for the more hydrophobic of two S. epidermidis strains having different surface properties; conversely, the more hydrophilic one adhered better to the heparinized polymer. Modification of polymer surfaces can thus result in complex effects depending on bacterial surface properties, among other factors.

Bacterial adherence to surfaces is an extremely complex, step-by-step process. The bacterial surface contains hydrophobic as well as hydrophilic sites, the latter including both positively and negatively charged groups. Various physicochemical approaches have been used to investigate the interactions of bacteria with materials to understand how surface treatments can alter the ability of materials to bind bacteria. Van der Mei et al. studied the deposition of six coagulase-negative staphylococci strains on negatively charged PMMA and on a positively charged PMMA copolymer, while keeping the effect of hydrophobic interactions constant. As expected, deposition decreased with increasing electrostatic repulsion between bacteria and the negatively charged polymer. Finally, the production of capsular polysaccharide or slime increases both the hydrophilicity and the negative charge of the bacterial surface, thereby affecting the adhesive properties of S. epidermidis. In our work, owing to the uncharged character of silicone, the strong adherence of bacteria to this highly hydrophobic polymer was not counteracted by electrostatic repulsion. Other materials, made of acrylate or methacrylate ester monomers, probably contain some free carboxyl groups and thus bear negative charges, as described for PMMA. Not only are these polymers, especially HEMA, less hydrophobic than silicone, but the presence of negative charges also results in electrostatic repulsion that proportionally reduces hydrophobic interactions between the bacterium surface and the hydrocarbon skeleton of the polymer. With hydrogel, the latter interactions would be further impeded because HEMA hydroxyl groups form hydrogen bonds with water molecules in the medium. Like HEMA, heparinized PMMA is covered with hydroxyl groups. The resultant decrease of hydrophobic interactions with the bacterial surface hampers the initial binding event. However, various kinds of polysaccharide chains are known to associate, either directly or through cation bridges, yielding gels. Such interactions cannot be excluded between heparin and the slime polysaccharide, and could explain why bacterial adherence to heparinized PMMA was stronger than to HEMA, because the latter material is not expected to associate with polysaccharides.

The lack of statistical significance between PMMA, heparinized PMMA, silicone, and hydrophilic acrylic (for bioluminescence only) could be explained by the low number of IOLs per group. Therefore, the power of the analysis may be limited, all the more so because the wide standard deviations must be taken into consideration.

Slight classification differences were noted between the two methods—above all, for hydrophilic acrylic. Different explanations are possible. Bioluminescence is a simple and rapid method to appreciate the number of bacteria attached to surfaces. It provides a very sensitive assay for ATP, from 10 to 10,000 times better than spectrophotometry, the detection limit of which is as low as 10⁻¹⁶ moles. According to many authors, the correlation between ATP concentration and the number of bacterial cells is quite satisfactory and the sensitivity of the method, which requires only very small quantities of reagents, is a way to reduce the cost greatly. The number of CFU is calculated from the amount of ATP through a linear regression curve, using logarithmic values of ATP and CFU. Despite this, the quantification of cellular ATP corresponds to a valuation of cell activity, but inside a biofilm, not all the bacteria have the same metabolic activity, because bacterial metabolism is qualitatively modified when bacteria are fixed. The bacteria located within deeper layers of the biofilm expressed reduced metabolic activity. This is probably why bioluminescence expressed lower values than SEM did. To represent reality, results were expressed as picomolar of ATP per 100 mm² instead of CFU per 100 mm².

Moreover, SEM presents three advantages compared with bioluminescence. It allows counting all bacteria irrespective of their metabolic activity level, and data showed lower standard

### Table 2. Bacterial Adherence as Measured by Bioluminescence and SEM

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<tr>
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<th>Hydrogel</th>
<th>Hydrophilic Acrylic</th>
<th>PMMA</th>
<th>Heparinized PMMA</th>
<th>Silicone</th>
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<tbody>
<tr>
<td>Bioluminescence</td>
<td>0.17 ± 0.17</td>
<td>0.98 ± 0.92</td>
<td>1.35 ± 0.45</td>
<td>1.56 ± 1.06</td>
<td>1.65 ± 1.41</td>
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<tr>
<td>SEM</td>
<td>3.7 ± 5</td>
<td>27.3 ± 5</td>
<td>171.8 ± 8</td>
<td>188 ± 10</td>
<td>173.7 ± 9</td>
</tr>
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Data are mean ratios ± SD.

* P<0.05.

### Table 3. Bioluminescence: Probabilities of the Differences between the Types of IOLs According to the Mann-Whitney Nonparametric Test

<table>
<thead>
<tr>
<th>IOL</th>
<th>Hydrogel</th>
<th>Hydrophilic Acrylic</th>
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<tr>
<td>Hydrogel</td>
<td>—</td>
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</tr>
<tr>
<td>Hydrophilic Acrylic</td>
<td>0.010 *</td>
<td>0.522</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>PMMA</td>
<td>0.008 *</td>
<td>0.672</td>
<td>0.643</td>
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<tr>
<td>Heparinized PMMA</td>
<td>0.009 *</td>
<td>0.568</td>
<td>0.663</td>
<td>0.558</td>
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<tr>
<td>Silicone</td>
<td>0.009 *</td>
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* P<0.05.
deviations. This is partly due to the high sensitivity of bioluminescence, as well as to the fact that values are near the limit of detection with bioluminescence. Last, SEM observations allow three-dimensional details to be visualized on a sample surface, with the direct observation of adhering bacteria and biofilm morphologic characteristics. Thus, even if differences between PMMA, heparinized PMMA, and silicone were not statistically significant, SEM observations demonstrated that the behavior of attached bacteria depended on the IOL surface by showing two different types of adhesion. For hydrogel, hydrophilic acrylic and heparinized PMMA IOLs, bacteria seemed to be isolated (Figs. 1, 2, 5). In contrast, clusters of bacteria were seen on PMMA and silicone IOLs (Figs. 3, 4). These bacteria were accumulated on the lens surface as microcolonies, meaning that the bacteria probably secreted a layer of adhesive material (slime) and were literally embedded. Biofilm formation is commonly thought to be a two-step process, involving various specific factors: adhesion of cells to a solid substrate followed by cell-to-cell adhesion. The first phase is mediated by nonspecific physicochemical forces and/or the capsular polysaccharide/adhesin (noted classically PS/A) and/or several surface proteins. PS/A was described as mediating primary attachment to silastic catheter surfaces. Biofilm formation is commonly thought to be a two-step process, involving various specific factors: adhesion of cells to a solid substrate followed by cell-to-cell adhesion. The first phase is mediated by nonspecific physicochemical forces and/or the capsular polysaccharide/adhesin (noted classically PS/A) and/or several surface proteins. PS/A was described as mediating primary attachment to silastic catheter surfaces. The second phase is mediated by the bacterial production of a polysaccharide glycolycal (slime) on the IOL surface, including a polysaccharide intercellular adhesin (noted classically PIA). In 1992, Mack et al. demonstrated the implication of PIA for the accumulation of multilayered biofilm. Our observations with cluster formation are totally in keeping with the observations of Mack et al. in 1994. Two wild strains were able to produce clusters, whereas mutants unable to produce PIA were impaired in the accumulative phase of biofilm production, and isolated bacteria were observed. A recent study has shown that the intercellular adhesion (ica) locus of <i>S. epidermidis</i> encodes production of both PS/A and PIA. It so happens that the strain under study carried the <i>ica</i> locus. Thus, the formation of clusters of bacteria on IOLs, as opposed to isolated bacteria, could present an additional step in forming a biofilm, and consequently in colonizing a biomaterial. Therefore, PMMA and silicone materials could facilitate bacterial colonization and biofilm production, more so than hydrogel, hydrophilic acrylic, and heparinized PMMA. Another hypothesis is that bacterial colonization is only delayed with hydrophilic IOLs. However, host defenses and/or antibiotics are more efficient on bacteria simply attached than on bacteria embedded within a layer of slime, because they have trouble penetrating the biofilm and killing the embedded bacteria. Therefore, even if colonization of these IOLs is only delayed, host defenses are given more time to react. There is little information in the literature concerning the relationship between IOL materials and endophthalmitis. In a retrospective study, Montan et al. showed that implanting a heparinized PMMA IOL significantly reduces the risk of endophthalmitis compared to using PMMA or silicone IOLs. Even if these results are in complete keeping with our present hypothesis, only further epidemiologic studies will help to confirm and complete this relationship.

The results of this study suggest that the risk of endophthalmitis caused by <i>S. epidermidis</i> after cataract extraction and IOL implantation may be lower when IOLs made of less sticky material are used, such as hydrogel, and probably hydrophilic acrylic as well. However, the clinical relevance remains to be shown.

<table>
<thead>
<tr>
<th>Table 4. SEM: Probabilities of the Differences between the Types of IOLs according to the Mann-Whitney Nonparametric Test</th>
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<tbody>
<tr>
<td><strong>IOL</strong></td>
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<td>Hydrogel</td>
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*<i>P < 0.05</i>.

### References
