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COMMUNICATION

In-Vitro Study of Bacterial Adherence to Different Types of Intraocular Lenses

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ABSTRACT

The aim of this study was to determine the adherence of Staphylococcus epidermidis *to intraocular lenses made of five different biomaterials: polymethylmethacrylate (PMMA), heparinized PMMA, silicone, hydrophilic acrylic, and hydrogel. The extent of bacterial binding was measured by counting. The results were compared using a one-factor variance analysis. Adherence was weakest on hydrogel and strongest on the silicone polymer. Bacterial adherence to the implant surface must therefore depend on the hydrophobicity or hydrophilicity of the biomaterial.*

INTRODUCTION

Postoperative endophthalmitis is still one of the most feared complications of intraocular lens (IOL) implantation. It remains difficult to foresee and to diagnose, and represents a therapeutic emergency, because of its rapid evolution and bad prognosis. Nowadays, although endophthalmitis can be cured with a minimum of sequelae, an appreciable percentage of cases remain responsible for a definitive functional loss (50% of the patients recover visual acuity lower than 20/400), or even for anatomical eye loss (1).

Bacterial adherence on IOLs during their insertion is believed to represent an important factor in the pathogenesis of endophthalmitis and of pseudophakic chronic intraocular inflammations (2,3). Thus, one can hope to decrease endophthalmitis incidence by reducing the adherence of bacteria to intraocular implants, and mainly that of the most often involved germ, *Staphylococcus epidermidis* (4).

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The aim of this work was to study bacterial adherence to the different materials of which IOLs are composed.

MATERIALS AND METHODS

Materials

A study was carried out on 120 IOLs made of five different plastic materials: polymethylmethacrylate (PMMA) (30 lenses), heparinized (HSM or heparin-surface-modified) PMMA (30 lenses), silicone (18 lenses), hydrophilic acrylic (acrylate or methacrylate polymers) (18 lenses), and hydrogel (HEMA = hydroxy-ethyl-methacrylate or PHEMA = poly-HEMA) (24 lenses). Various firms in France produce these new sterile inserts (Table 1).

Strains

Herriot Hospital microbiology laboratory (Lyon, France) provided a *S. epidermidis* strain (laboratory collection no. 890 074) that produced a great amount of slime. The bacterial concentration was adjusted spectrophotometrically to 10^8 colony-forming units (CFU) per ml.

Methods

Complete lenses (including haptics) were incubated in the bacterial suspension for 1 hr at 37°C, with continuous shaking, before being washed three times in a phosphate-buffered saline solution (PBS buffer, pH 7.8) to eliminate non-adhering bacteria. Washed lenses were soaked in PBS buffer and bound bacteria were then dispersed by sonication at 45 kHz for 5 min using a Bransonic device. The resulting suspension was diluted and spread on a nutritive agar plate (Trypticase-Soja, bioMérieux, Marcy l'Étoile, France). Colonies were counted after a 24-hour incubation at 37°C; numbers of bacteria were expressed as CFU/ml. The area of each lens depends on its diameter, but also on its haptic shape and dioptric power; thus, IOL manufacturers gave the exact area of all studied implants, which varied from 47 to 113 mm^2. Results were expressed as $\log_{10}$ CFU per 100 mm^2. They were compared using a one-factor variance analysis test (ANOVA, Microsoft Excel 5).

RESULTS AND DISCUSSION

Bacterial adherence to IOLs made from five more or less hydrophobic materials was measured as described in Materials and Methods. During preliminary in-vitro studies, we checked that a 5-min sonication at 45 kHz did not affect bacterial viability, in keeping with previous results from Raskin et al. (5). Moreover, using microscopic examination, Abu El-Asrar et al. (6) have verified that this treatment removed all bound bacteria from the lenses. The incubation time of implants in the bacterial suspension was set as 1 hr only, because our preliminary studies based on results from Ludwika et al. (7) showed that the number of IOL-adherent bacteria reached its highest value from a 1 hr incubation onward.

The number of bound bacteria per unit area found on hydrogel, heparinized PMMA, acrylic, PMMA, and finally on the silicone polymer, went in increasing order. If compared with those measured on heparinized PMMA, acrylic, or untreated

Table 1

Characteristics of the IOLs

Material	Manufacturer	Model Number	Style of Haptics
PMMA	Allergan, Paris	PS53ANB	1-piece
PMMA	Chiron, Lyon	MIC6	1-piece
Heparinized PMMA	Pharmacia, Paris	728C	1-piece
Silicone	Corneal, Annecy	SM575	3-piece
Silicone	Allergan, Paris	SI30NB	3-piece
Acrylic	Corneal, Annecy	ACR6D	3-piece
Hydrogel	Alcon, Paris	Iogel	Plate

PMMA, these counts were clearly lower on hydrogel and much greater on silicone (Fig. 1). Consequently, the average $\log_{10}$ CFU per unit area measured on one material was compared with that found for each of the four other materials, respectively, according to the ANOVA test. As could be expected from Fig. 1, the differences obtained between hydrogel and each of the four other materials were statistically significant ($p < 0.01$). When comparing silicone with each of the four other materials, these differences were also statistically significant ($p < 0.01$). On the contrary, the pairwise comparison of PMMA, heparinized PMMA, and acrylic did not give any significant difference.

Coagulase-negative staphylococci are currently recognized as important etiological agents of endophthalmitis following the implantation of IOLs (8). Since the treatment of this disease is difficult and sometimes inefficient, modification of the polymer lens surface might represent a promising approach intended to alter bacterial adherence, which is the first step in the colonization of a territory (9).

In contrast to results from Hogt et al. (10), who reported in 1986 that bacterial adherence to methacrylate polymers was inversely correlated to their respective hydrophobicities, most authors have concluded that hydrophobicity is an important promoting factor of bacterial binding (11–13). Moreover, Dilly and Holmes Sellors in 1989 (3) and Cusumano et al. in 1991 (2) showed in vivo that stronger adherence was observed on silicone than on PMMA. We reached the same conclusion, since silicone—the most hydrophobic polymer—gave the highest number of adherent bacteria in a statistically significant way.

Likewise, Dankert et al. (14) found in 1986 that a hydrophobic bacterial strain adhered more easily on a hydrophobic surface than did a hydrophilic one, whereas the binding of bacteria to a hydrophilic surface was weaker whatever the strain, hydrophobic or hydrophilic. Later, Jansen and Peters (8) proved in 1991 that adherence was weaker on the most hydrophilic surfaces, as suggested previously by Kristinsson in 1989 (15). These observations are in keeping with our results: hydrogel, the most hydrophilic material, harbored a significantly lower

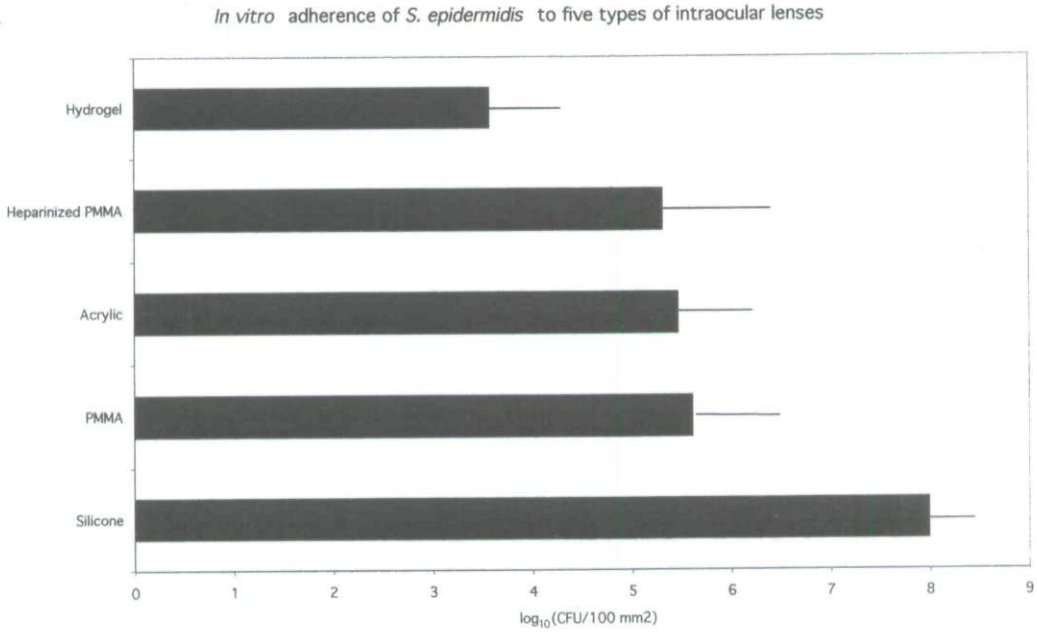

Figure 1. Bacterial adherence as measured from in-vitro counts. The number of lens-bound bacteria, expressed as $\log_{10}$ CFU per 100 mm^2, clearly increased from the hydrogel-made IOLs to the silicone-made IOLs; it was lowest on hydrogel and highest on silicone. There was no significant difference between the three other materials, which gave intermediate bacterial counts. For conditions, see text. Error bar = standard deviation.

number of bound bacteria than other polymers, in accordance with the findings of Ng et al. (4).

With heparinized PMMA, the situation was much less clear-cut. Some previous studies brought out the fact that heparinization reduced staphylococcal adherence (16–18). Whereas, on the one hand, the long hydrophilic heparin chains that were fixed on the lens surface could capture neighboring water molecules, forming a hydrophilic boundary layer all around the lens, which could in turn decrease cellular and bacterial binding (16,18), on the other hand, heparin bears negative charges that might promote adherence inhibition (6,18). In fact, according to our study, somewhat less bacteria adhered to heparinized PMMA than to silicone, untreated PMMA, and acrylic, although the difference was not statistically significant. Perhaps the coating of PMMA by heparin chains is not dense enough in places; thus, some hydrophobic areas could still be exposed on the outer layer.

It is worth noting that factors other than the material—e.g. the bacterial strain—could affect adherence. For instance, Tetz et al. (17) proved in vitro that a *S. epidermidis* strain adhered less easily on heparinized than on untreated PMMA, but they failed to obtain the same result with another strain, thus suggesting a large disparity among bacteria, as far as heparinized implants are concerned. Similarly, when comparing two *S. epidermidis* strains having different surface properties, Schmidt et al. (19) and Schloricke et al. (20) found that the more hydrophobic strain bound to a lesser extent to heparinized PMMA than to untreated PMMA or silicone, whereas the more hydrophilic one adhered more strongly on the heparinized polymer. Therefore, the modification of polymer surfaces can result in rather variable effects, depending—among other factors—on the bacterial surface composition and properties.

Bacterial adherence to surfaces involves extremely complex interactions that are unlikely to fall within the scope of one generally valid description. The bacterial surface contains hydrophobic as well as hydrophilic titratable sites, the latter including both positively and negatively charged groups, (9,21). The initial binding event is thus governed by the balance between three fundamental physicochemical forces: van der Waals, electrostatic, and acid–base interactions. Various analytical methods must be used to clarify the mechanisms involved in bacterial adherence in order to understand how an appropriate surface treatment can modify the interactions of bacteria with materials. For a review, see (9).

During microbial attachment to surfaces, free energies of adherence are almost always negative because van der Waals forces involved in hydrophobic interactions are essentially attractive. These forces are further modulated by either repulsive or attractive electrostatic forces and by acid–base interactions. The net charge of a bacterium surface depends both on its isoelectric point and on the pH of the surrounding medium. Moreover, electrostatic forces decrease with increasing ionic strengths. Generally, electrostatic repulsion usually dominates under low ionic strength conditions, since both surfaces often bear negative charges (9). However, the picture may be even more complex, since the cell surface hydrophobicity relates to acid–base interactions (22).

Adherence of staphylococci to mucosal cells was reported to involve mainly lipoteichoic acid (LTA) rather than wall proteins; the essential role of LTA fatty acids suggested that such an adherence was mediated by hydrophobic interactions (23). Electrostatic forces must also be taken into account. While keeping the influence of hydrophobic interactions constant in a parallel plate flow chamber, Van der Mei et al. (24) studied the deposition of six coagulase-negative staphylococci on PMMA (which is negatively charged) and on a positively charged PMMA copolymer. As expected, deposition efficiencies decreased with increasing electrostatic repulsion between bacteria and the negatively charged polymer. Finally, the production of capsular polysaccharide or slime (25) by the strain used was expected to increase both the hydrophilicity and the negative charge (26) of the bacterium surface, thereby affecting the adhesive properties of *S. epidermidis* (27).

In our work, owing to the uncharged character of the polymer, the strong adherence of bacteria to the highly hydrophobic silicone could not be counteracted by electrostatic repulsion. Other materials, built from acrylate or methacrylate ester monomers, were expected to contain some free carboxyl groups and thus to bear negative charges, as described for PMMA (24). Not only are these polymers, and especially HEMA, less hydrophobic than silicone, but the presence of charged groups results in electrostatic forces that alleviate hydrophobic interactions between the bacterium surface and the

hydrocarbon skeleton of the polymer. With hydrogel, the latter interactions would be further impeded because of the propensity of HEMA hydroxyl groups to form hydrogen bonds with water molecules in the medium.

In fact, PMMA bears some resemblance to HEMA after heparinization, insofar as it becomes covered with hydroxyl groups, which leads to decreased hydrophobic interactions with the bacterial surface. This prevents the initial binding event. However, various kinds of polysaccharide chains are known to associate together, either directly or through cation bridges, yielding gels (28). The possibility of such interactions between heparin and the slime polysaccharide must not be excluded, and could possibly explain why bacterial adherence to heparinized PMMA was stronger than to hydrogel. In any case, the interest of a hydrophilic polymer surface that can avoid the development of bacterial colonies, and hence prevent endophthalmitis, remains to be proven in vivo. Furthermore, the influence of the surrounding medium makes it difficult to extrapolate in-vitro results to a clinical situation. At last, in-vitro adherence can be as well influenced by incubation times (29).

To sum up, since germ-binding onto implants, when introduced into the eye, has been recognized as an outstanding factor in the etiopathology of post-surgery endophthalmitis, one can hope that the incidence of this disease will decrease once the adherence of germs—especially that of staphylococci—to IOLs is loosened. To our knowledge, no study has been published so far concerning the comparison of bacterial adherence to five commercialized kinds of IOLs (120 IOLs). Adherence was found to be weakest on hydrophilic materials such as hydrogel, and strongest on the most apolar ones such as silicone. The status of heparinized PMMA was not as well delineated and will require further work with strains differing in cell surface hydrophobicity.

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