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ORIGINAL ARTICLES

Adhesion of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* to Silicone–Hydrogel Contact Lenses

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ABSTRACT: *Purpose.* The purpose of this study is to compare the adhesion capabilities of the most important etiologic agents of microbial ocular infection to the recently available silicone–hydrogel lenses with those to a conventional hydrogel lens. *Methods. In vitro* static adhesion assays of *Pseudomonas aeruginosa* 10,145, *Staphylococcus epidermidis* 9142 (biofilm-positive), and 12,228 (biofilm-negative) to two extended-wear silicone–hydrogel lenses (balafilcon A and lotrafilcon A), a daily wear silicone–hydrogel lens (galyfilcon A) and a conventional hydrogel (etafilcon A) were performed. To interpret the adhesion results, lens surface relative hydrophobicity was assessed by water contact angle measurements. *Results. P. aeruginosa* and *S. epidermidis* 9142 exhibited greater adhesion capabilities to the extended wear silicone–hydrogel lenses than to the daily wear silicone– and conventional hydrogel lenses ($p < 0.05$). No statistical differences were found between the adhesion extent of these strains to galyfilcon A and etafilcon A. The biofilm negative strain of *S. epidermidis* adhered in larger extents to the silicone–hydrogel lenses than to the conventional hydrogel ($p < 0.05$), but in much lower amounts than the biofilm-positive strain. The water contact angle measurements revealed that the extended wear silicone–hydrogel lenses are hydrophobic, whereas the daily wear silicone– and conventional hydrogel lenses are hydrophilic. *Conclusions.* As a result of their hydrophobicity, the extended wear silicone–hydrogel lenses (lotrafilcon A and balafilcon A) may carry higher risk of microbial contamination than both the hydrophilic daily wear silicone–hydrogel lens, galyfilcon A and the conventional hydrogel lens, etafilcon A. (Optom Vis Sci 2005;82:446–450)

Key Words: silicone–hydrogel contact lenses, bacterial adhesion, hydrophobicity, *P. aeruginosa, S. epidermidis*

Conventional soft lenses based on polyhydroxyethyl methacrylate (p-HEMA) are still the most popular type of contact lenses. These lens materials are copolymers of HEMA and other hydrophilic monomers such as N-vinyl pyrrolidine (NVP) and methacrylates that possess a wide range of water content. The water content is usually above 38 wt%, which contributes to the softness and comfort of these lenses. However, the oxygen permeability of these lenses is limited by the water phase restricting their wearing schedule. The introduction of silicone-containing hydrogel contact lenses having the same comfort and significant higher oxygen permeabilities than conventional hydrogel has resulted in a new generation of soft contact lenses. The high oxygen permeability on account of the Siloxane component makes it possible to wear these lenses on a continuous basis for up to 30 days.[1] Recently, the U.S. Food and Drug Administration approved a new silicone–hydrogel lens for daily wear (galyfilcon A) that combines the high oxygen transmissibility nature of a silicone–hydrogel with the great wettability and flexibility of a conventional hydrogel.[2]

One of the main problems associated with contact lenses is microbial contamination of the lens surface. Although the extended wear contact lens reduces the frequency of handling and thus the risk of contamination, no protection from infection by regular cleaning and disinfection is provided. Although the estimated risk of the incidence of silicone–hydrogel lens-associated keratitis is one in 15,800 patients years,[3] which is approximately 30 times lower than for conventional hydrogels,[4] this fact should not be ignored.[5]

Adhesion of bacteria, notably *Pseudomonas aeruginosa* and *Staphylococci* strains, to contact lenses is considered a primary risk factor of serious corneal problems.[6,7] The contact lenses provide a suitable substratum for bacterial adherence and biofilm formation,[8] supplying an inoculum of organisms in prolonged contact with the cornea. Additionally, the corneal interaction with the contact lens can overwhelm the protective mechanisms of the cornea, increasing the ability of microbial cells to adhere to the cornea and progress to microbial keratitis.[9] The risks associated with silicone–hydrogel lenses, regarding microbial contamination, have not been fully evaluated. In this work, the relative adhesion capability of the most important etiologic agents of microbial ocular infection (*P. aeruginosa* and *S. epidermidis*) to the recently available silicone–hydrogel lenses (lotrafilcon A, balafilcon A, and galyfilcon A) versus a standard hydrogel lens (etafilcon A) was studied.

MATERIALS AND METHODS

Contact Lenses

The silicone–hydrogel lenses used in this study lotrafilcon A (Focus Night & Day; CIBA), balafilcon A (PureVision; Bausch & Lomb), and galyfilcon A (Acuvue Advance, with HYDRACLEAR; Johnson & Johnson) were kindly provided by the manufacturers as well as the HEMA lens etafilcon A (Acuvue; Johnson & Johnson). The contact lenses properties are summarized in Table 1.

Artificial Tears

Artificial tears were prepared with 1.4 g of polyvinyl alcohol (Sigma-Aldrich) and 0.6 g of povidone (Sigma-Aldrich) in 100 mL of a saline solution (0.9% NaCl). The pH of this solution was adjusted to 7.5 with NaOH and sterilized by vacuum filtration through a 0.2-μm filter. Artificial tears were made with the purpose of reproducing physicochemical properties of natural tears, namely pH, ionic strength, and viscosity.

Viability tests based on CFU determinations of bacterial suspensions incubated in artificial tear and a control saline solution (0.9%) demonstrated that artificial tears do not affect the viability of the assayed bacterial strains (data not shown).

Bacterial Strains and Growth Conditions

The strains used in this study were a biofilm-negative *S. epidermidis* ATCC 12,228,[10,11] a biofilm-positive *S. epidermidis,* 9142 and *P. aeruginosa* ATCC 10,145. *S. epidermidis* 9142 is a known producer of the major surface polysaccharide promoting coagulase-negative Staphylococci adherence and biofilm formation, referred to as either polysaccharide intercellular adhesin (PIA) or by its chemical composition, poly-N-acetyl glucosamine (PNAG). This strain was provided by Dr. Gerald B. Pier, Harvard Medical School, Boston. Strains of S. *epidermidis* ATCC 12,228 and *P. aeruginosa* ATCC 10,145 were obtained from the American Type Culture Collection. All strains were stored at -70°C on 25% glycerol.

Staphylococci strains and *P. aeruginosa* were incubated in 10 mL of TSB tryptic soy broth (TSB) during 24 hours at 37°C. After this period, 100 μL of the culture suspension were transferred to 50 mL of fresh TSB and incubated for 18 hours at 37°C to obtain a midexponential growth culture. Cells were harvested by centrifugation (15 minutes, 5000 g) and washed two times with artificial tears.

Adhesion Assays

The method used to assess bacterial adhesion to contact lenses was the static adhesion assay. This method consisted in immersing each contact lens, with the convex side up, in 1 mL of a cell suspension (5×10^8 CFU/mL) prepared in artificial tears and placed in a well of a 24-well tissue culture plate (Sarstedt). The tissue culture plate was incubated for 2 hours at 37°C and after this period, each contact lens was carefully removed from the well with a tiny forceps and washed three times by immersing the lens in clean artificial tears for 15 seconds. This washing step was carefully performed to remove only the cells that were suspended in the liquid interface formed along the surface and to minimize cell detachment from the surface.

After the adhesion assay, two opposite edges of each contact lens were cut to flatten the surface to be mounted on a microscope slide with the correspondent convex side up. Cell enumeration was performed using a phase contrast microscope coupled to a 3-CCD video camera that acquires images at a magnification of 1622 × and 20 images were randomly taken from each contact lens. Cells were enumerated using an image analysis system (SigmaScan Pro5 SPSS). The adhesion experiments were done in triplicate and repeated twice.

Contact Angle Measurements

Relative lens surface hydrophobicity was determined by measurements of water contact angles. Before the measurements, contact lenses were immersed in artificial tears for 30 minutes. The excess of liquid was then removed by gently tapping the side of the lens on a filter paper. Contact lenses were then cut into quarters

TABLE 1.
Summary of contact lens properties

Category	Name, material	Manufacturer	FDA group	Water content (wt.%)	Oxygen permeability (Dk barrera)
Silicon-based	Focus Night & Day, lotrafilcon A	CIBA	I	24	140
	PureVision, balafilcon	Bausch & Lomb	III	36	91
	Acuvue Advance,galyfilcon A	Johnson & Johnson	I	47	60
p-HEMA-based	Acuvue, etafilcon A	Johnson & Johnson	IV	58	28

[a] $\times 10^{-11}$ cm mL O_2/s mL mm Hg.

and each quarter mounted on a microscope slide. Measurements of advancing type water contact angles were carried out on the convex side of a contact lens quarter using the apparatus OCA 20 (Dataphysics). The measurements were performed immediately after cutting the contact lens to avoid lens dehydration and at 25°C. These measurements were repeated eight times per contact lens material.

Statistics

The data obtained was analyzed using a statistical program, SPSS (Statistical Package for the Social Sciences). One-way analysis of variance with Tukey test was used to compare the number of adhered cells for each contact lens type and for each strain. All tests were performed with a confidence level of 95%.

RESULTS

The number of cells of *P. aeruginosa* and *S. epidermidis* (ATCC 12,228 and 9142) attached to the three silicone–hydrogel contact lenses and to the conventional hydrogel lens are present in Figure 1. *S. epidermidis* 12,228 adhered in larger extent to the silicone–hydrogel contact lenses than to the conventional hydrogel ($p < 0.05$). Conversely, no statistical differences were found between the adhesion of *S. epidermidis* 9142 and *P. aeruginosa* to galyfilcon A and etafilcon A, although the adhesion of these two strains to lotrafilcon A and balafilcon A occurred in greater amounts ($p < 0.05$).

Comparing the adhesion behaviors of the three strains, it was found that the number of adhered cells of *P. aeruginosa* to etafilcon A was significantly higher than that of *S. epidermidis* 12,228 ($p = 0.002$) and *S. epidermidis* 9142 ($p = 0.005$). There were no statistical differences in the adhesion extents of all strains to galyfilcon A. Considering the adhesion to lotrafilcon A and balafilcon A, the number of adhered *S. epidermidis* 12,228 was significantly lower than the number of adhered *S. epidermidis* 9142 ($p = 0.01$ and $p = 0.045$, respectively, to each contact lens) and the number of adhered *P. aeruginosa* 10,145 ($p = 0.003$ and $p = 0.004$, respectively, to each contact lens).

Figure 2 presents the values of the water contact angles measured on the lens surfaces. According to van Oss and Giese,[12] material surfaces can be considered hydrophobic if the water contact angle is higher than 50°. Considering this notation balafilcon A and lotrafilcon A are hydrophobic, whereas galyfilcon A and etafilcon A are hydrophilic.

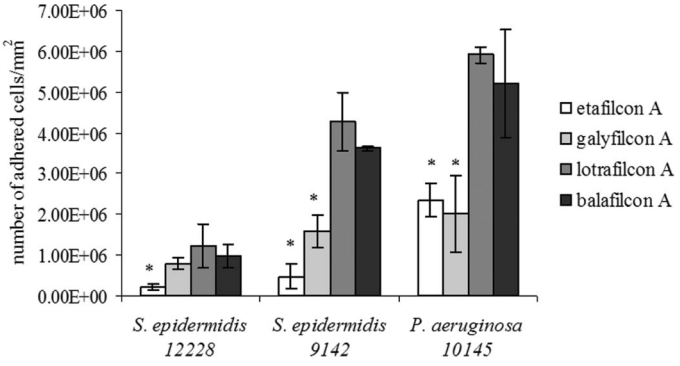

FIGURE 1.

Number of adhered cells of *Pseudomonas aeruginosa* 10,145, *Staphylococcus epidermidis* 9142, and *S. epidermidis* 12,228 per mm² to each type of contact lens. The asterisk represents the statistical differences.

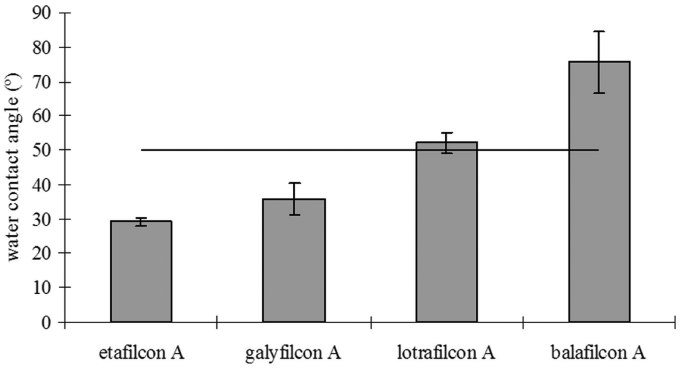

FIGURE 2.

Water contact angle (ϑ) formed on silicone–hydrogel lenses (galyfilcon A, lotrafilcon A, and balafilcon A) and conventional hydrogel (etafilcon A). Hydrophobic surfaces have water contact angles above 50°. Bars represent the standard deviations.

DISCUSSION

In this study, adhesion to contact lenses was performed using a static slide method. This methodology of assessing adhesion has been controversial because of the use of washing steps necessary to remove nonadherent and loosely adherent cells.[13] It has been demonstrated that the passage of an air–liquid interface on adhered bacteria can remove some of the adhered bacteria.[14] However, when in the presence of a more hydrophilic substratum or for higher interface passage speeds, this effect is attenuated.[15] Cerca et al.[16] studied the adhesion of 11 clinical strains of *S. epidermidis* to acrylic and glass surfaces using several different washing procedures and demonstrated that when using hydrophilic glass, no effect was observed on the passage of the air–liquid interface. In the case of hydrophobic surfaces, that effect was sometimes observed but attenuated by rapid washing to minimize the time of exposure of the adherent cells to the air–liquid interface.

The most common approach to enumerate adherent bacteria relies on the removal of the organisms from the lens surface followed by viable cells culturing. The method used in this study is based on the direct enumeration of adhered cells.[17] This technique is advantageous over other methods that use vortexing or sonication to remove adhered cells in ensuring that all adhered bacteria are quantified. Additionally, on account of microbial aggregation (very common on *Staphylococci* species), colony-forming units usually underestimate the number of cultivable bacteria.

The incorporation of silicone into a hydrogel polymer gives the advantage of a high oxygen transmissibility, but the disadvantage of decreased hydrophilicity.[18] To render the surface hydrophilic, techniques incorporating plasma into the surface of the lens have been developed. In the case of lotrafilcon lenses, they are permanently modified in a gas plasma-reactive chamber to create a continuous hydrophilic surface.[1,19] However, the water contact angles measured on the surface of this lens revealed a hydrophobic surface (Fig. 2). In the case of balafilcon, the lens surface is treated in a gas

plasma-reactive chamber, which transforms the silicone components on the lenses surface into hydrophilic silicate compounds, resulting in the formation of "silicate islands."[1,19] Between this, silicate islands are hydrophobic areas, which may explain the high water contact angle formed on the surface of this lens (Fig. 2) and thus its higher hydrophobicity compared with lotrafilcon. Galyfilcon A has no surface modification, but incorporates a moisture-rich internal wetting agent brandnamed HYDRACLEAR, based on PVP (polyvinyl pyrrolidone), that provides a hydrophilic layer at the surface of the material, which reduces the degree of hydrophobicity.[2]

The two extended wear silicone–hydrogel lenses have a surface hydrophobicity higher than that of conventional hydrogel lens and the daily wear silicone–hydrogel lens. These differences in surface hydrophobicity may explain the differences found in bacterial adhesion. Many studies have suggested that hydrophobic surfaces are more prone to pathogens adhesion than hydrophilic ones.[15,20] Beattie et al.[21] studied *Acanthamoeba* attachment to a silicone–hydrogel lens (balafilcon A) and conventional hydrogel contact lenses and concluded that balafilcon A is more prone to bacterial adhesion. These authors suggested that the high levels of attachment found in silicone–hydrogel lenses may be the result of the inherent property of the polymer or of the surface treatment procedure that originates in areas of hydrophobic material unoxidized after treatment.

In the present study, it was found a significant higher extent of adhesion of *P. aeruginosa* to the silicone–hydrogel lenses than to the conventional hydrogel lens (Fig. 1), with the exception of galyfilcon A. Willcox et al.[22] also found an increased capability of *P. aeruginosa* to adhere to silicone–hydrogel balafilcon A when compared with the adhesion to conventional hydrogels. Conversely, Borazjani et al.[23] found no significant differences between the adhesion of *P. aeruginosa* to silicon–hydrogel balafilcon A and etafilcon A. These contradictory results may be the result of the different bacterial strains used and growth conditions used. Several authors have reported that the extent of *P. aeruginosa* adherence is strain-dependent and influenced by growth stage and media.[22,24-26]

The ability of *S. epidermidis* 9142 to adhere to the hydrophobic silicone–hydrogel lotrafilcon A and balafilcon A was also greater than to the hydrophilic etafilcon A and hydrophilic silicone–hydrogel galyfilcon A, reinforcing the idea that hydrophobic silicone lens are more prone to bacterial adhesion.

The same conclusions could not be drawn for the strain 12,228, which is considered a biofilm-negative strain. Despite of being hydrophilic, galyfilcon A was equally prone to *S. epidermidis* 12,228 adhesion as the other hydrophobic silicone–hydrogel lenses. However, because this strain exhibited very low adherence capabilities, the differences observed in the extents of adhesion were not statistically relevant. Garcia-Saenz et al.[27] also reported low adhesion extents of a biofilm positive strain of *S. epidermidis* to contact lenses. The low adherence ability of this strain, compared with the biofilm-positive 9142, may be related to the absence of the *ica* operon in strain 12,228.[11] It is well documented that genes contained in the *ica* locus are responsible for the production of poly-N-acetyl-glucosamine (PNAG) and that PNAG is important for biofilm formation and adhesion to catheters.[28,29]

It must be stressed that the adhesion studies were carried out on the native polymer of contact lenses. However, it should be considered that *in situ* contact lenses become rapidly conditioned with the tear film proteins and mucins, which may modulate bacterial adhesion either by altering lens surface properties like hydrophobicity or by inducing the establishment of specific interactions between tear molecules and microbial cell receptors.[25] Nevertheless, this study may provide an indication of the likely transference of bacterial cell from the wearer's fingers to the contact lenses surfaces. On the other hand, Borazjani et al.[23] found no marked differences in the adhesion of *P. aeruginosa* to worn and unworn silicon–hydrogel lenses, suggesting that these lens surface properties were not affected by 6 to 7 days extended wear and thus by the presence of tear film molecules.

Summarizing, this *in vitro* adhesion studies revealed that silicone–hydrogel are more prone to bacterial colonization than conventional hydrogel lens. The exception was found for galyfilcon A that exhibited the same degree of adhesion of etafilcon A as a result of its low hydrophobicity. On the basis of this data, it could be speculated that the risks associated with silicone–hydrogel extended wear, when regarding microbial adhesion, would be higher than conventional hydrogel and daily wear silicone–hydrogel. However, the increased oxygen transmissibility of silicone–hydrogel lenses reduces corneal hypoxia and diminishes tissue damage, leading to reduced bacterial binding to corneal epithelium cells.[30] Thus, the drawbacks of lens colonization are minimized and the safety of extended wear of this type of lens is improved.

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REFERENCES

1. Tighe B. Silicone hydrogel materials—how do they work? In: Sweeney DF, ed. Silicone Hydrogels: The Rebirth of Continuous Wear Contact Lenses. Oxford: Butterworth Heinemann; 2000:1-21.
2. Steffen RB, McCabe KP. Finding the comfort zone with the newest silicone hydrogel technology. Contact Lens Spectrum, March 2004. Available at: http://www.clspectrum.com/article.aspx? article-&loc-archive\2004\march\vistakon\0304vistakon.htm. Accessed March 14, 2005.
3. Holden BA. Is continuous wear the only way to go? Clin Exp Optom 2002;85:123–6.
4. Lam DS, Houang E, Fan DS, Lyon D, Seal D, Wong E. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. Eye 2002;16:608–18.
5. Lee KY, Lim L. Pseudomonas keratitis associated with continuous wear silicone–hydrogel soft contact lens: a case report. Eye Contact Lens 2003;29:255–7.
6. Buehler PO, Schein OD, Stamler JF, Verdier DD, Katz J. The increased risk of ulcerative keratitis among disposable soft contact lens users. Arch Ophthalmol 1992;110:1555–8.
7. Leitch EC, Harmis NY, Corrigan KM, Willcox MD. Identification and enumeration of staphylococci from the eye during soft contact lens wear. Optom Vis Sci 1998;75:258–65.
8. Elder MJ, Stapleton F, Evans E, Dart JK. Biofilm-related infections in ophthalmology. Eye 1995;9:102–9.

9. Liesegang TJ. Contact lens-related microbial keratitis: part II: pathophysiology. Cornea 1997;16:265–73.
10. 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;26:1673–9.
11. Zhang YQ, Ren SX, Li HL. Genome-based analysis of virulence genes in a non-biofilm-forming Staphylococcus epidermidis strain (ATCC 12228). Mol Microbiol 2003;49:1577–93.
12. van Oss CJ, Giese RF. The hydrophilicity and hydrophobicity of clay minerals. Clays Clay Minerals 1995;43:474–7.
13. Bos R, van der Mei HC, Busscher HJ. Physico-chemistry of initial microbial adhesive interactions—its mechanisms and methods for study. FEMS Microbiol Rev 1999;23:179–230.
14. Gomez-Suarez C, Busscher HJ, van der Mei HC. Analysis of bacterial detachment from substratum surfaces by the passage of air-liquid interfaces. Appl Environ Microbiol 2001;67:2531–7.
15. Gómez-Suárez C, Noordmans J, van der Mei HC, Busscher HJ. Detachment of colloidal particles from collector surfaces with different electrostatic charge and hydrophobicity by attachment to air bubbles in a parallel plate flow chamber. Phys Chem 1999;1:4423–7.
16. Cerca N, Pier GB, Oliveira R, Azeredo J. Comparative evaluation of coagulase-negative staphylococci (CoNS) adherence to acrylic by a static method and a parallel-plate flow dynamic method. Res Microbiol 2004;155:755–60.
17. Azeredo J, Meinders J, Feijó J, Oliveira R. Determination of cell number and size of a population of *Pseudomonas fluorescens* by image analysis. Biotech Techniques 1997;11:355–8.
18. Tighe B. Silicone hydrogels: what are they and how should they be used in everyday practice? Optician 1999;218:31–32.
19. Lopez-Alemany A, Compan V, Refojo MF. Porous structure of Purevision versus Focus Night & Day and conventional hydrogel contact lenses. J Biomed Mater Res 2002;63:319–25.
20. Doyle RJ. Contribution of the hydrophobic effect to microbial infection. Microbes Infect 2000;2:391–400.
21. Beattie TK, Tomlinson A, McFadyen AK, Seal DV, Grimason AM. Enhanced attachment of acanthamoeba to extended-wear silicone hydrogel contact lenses: a new risk factor for infection? Ophthalmology 2003;110:765–71.
22. Willcox MD, Harmis N, Cowell, Williams T, Holden. Bacterial interactions with contact lenses; effects of lens material, lens wear and microbial physiology. Biomaterials 2001;22:3235–47.
23. Borazjani RN, Levy B, Ahearn DG. Relative primary adhesion of Pseudomonas aeruginosa, Serratia marcescens and Staphylococcus aureus to HEMA-type contact lenses and an extended wear silicone hydrogel contact lens of high oxygen permeability. Contact Lens Ant Eye 2004;27:3–8.
24. Thuruthyil SJ, Zhu H, Willcox MD. Serotype and adhesion of Pseudomonas aeruginosa isolated from contact lens wearers. Clin Exp Ophthalmol 2001;29:147–9.
25. Bruinsma GM, Rustema-Abbing M, de Vries J, Stegenga B, van der Mei HC, van der Linden ML, Hooymans JM, Busscher HJ. Influence of wear and overwear on surface properties of etafilcon A contact lenses and adhesion of Pseudomonas aeruginosa. Invest Ophthalmol Vis Sci 2002;43:3646–53.
26. Cowell BA, Willcox MD, Herbert B, Schneider RP. Effect of nutrient limitation on adhesion characteristics of Pseudomonas aeruginosa. J Appl Microbiol 1999;86:944–54.
27. Garcia-Saenz MC, Arias-Puente A, Fresnadillo-Martinez MJ, Paredes-Garcia B. Adherence of two strains of Staphylococcus epidermidis to contact lenses. Cornea 2002;21:511–5.
28. McKenney D, Hubner J, Muller E, Wang Y, Goldmann DA, Pier GB. The ica locus of Staphylococcus epidermidis encodes production of the capsular polysaccharide/adhesin. Infect Immun 1998;66: 4711–20.
29. Maira-Litran T, Kropec A, Goldmann D, Pier GB. Biologic properties and vaccine potential of the staphylococcal poly-N-acetyl glucosamine surface polysaccharide. Vaccine 2004;22:872–9.
30. Ren DH, Petroll WM, Jester JV, Ho-Fan J, Cavanagh HD. The relationship between contact lens oxygen permeability and binding of Pseudomonas aeruginosa to human corneal epithelial cells after overnight and extended wear. CLAO J 1999;25:80–100.

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