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# Bacterial Transmission from Contact Lenses to Porcine Corneas: An Ex Vivo Study

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**PURPOSE.** To quantify the transmission to ex vivo porcine eyes of *Staphylococcus aureus* 835 and *Pseudomonas aeruginosa* 3 from three types of contact lenses— one daily wear and two extended wear— differing in hydrophobicity and roughness.

**METHODS.** One daily wear lens (etafilcon) and two extended-wear lenses (one lotrafilcon A and one balafilcon A) were inoculated in a bacterial suspension for 30 minutes and then placed on ex vivo porcine eyes. After 16 hours of contact between lens and eye, confocal laser scanning microscopy was used to determine the number of bacteria on the lens and cornea for the calculation of transmission percentages.

**RESULTS.** Transmission percentages were significantly different for both bacterial strains from an etafilcon A lens and balafilcon A lens ($P = 0.006$ and 0.04, respectively). Percentages varied from 51% to 68% for the hydrophobic *P. aeruginosa* and from 54% to 82% for the hydrophilic *S. aureus* strain, depending on the contact lens involved. Both strains were transferred the least from the most hydrophilic and roughest lens made of lotrafilcon A, although the difference was only statistically significant for *S. aureus*.

**CONCLUSIONS.** Bacterial transmission to the porcine cornea differed in the various types of contact lenses and was least in the hydrophilic and rough lens type. (*Invest Ophthalmol Vis Sci.* 2005;46:2042–2046) DOI:10.1167/iovs.04-1401

Contact lens (CL) wear is the highest risk factor for the development of microbial keratitis in the Western world.[1] This inflammation of the cornea is a serious adverse event caused by bacteria and is potentially sight-threatening if not treated properly. Microbial keratitis can be considered a public health problem, because CLs are increasingly popular for cosmetic purposes, such as replacement of spectacles or adaptation of eye color, and they are used for therapeutic reasons as well.[2,3] Approximately two thirds of the isolated bacteria from CL-associated microbial keratitis are Gram negative, notably *Pseudomonas aeruginosa*, but Gram-positive bacteria such as *Staphylococcus aureus* are also frequently reported to be causative organisms.[4]

Contact lens wearers are prone to development of microbial keratitis because lenses are a potential means of transport of microorganisms to the cornea. Contact lenses CLs require occasional handling, for instance for cleaning or the relief of discomfort. During this handling, the CLs come into contact with contaminated objects, such as hands or lens storage cases, resulting in bacterial contamination and adhesion to the CLs.[5,6] When a lens is put onto the eye, bacteria are transferred to the corneal epithelium, potentially inducing an infection.[7,8]

Bacterial adhesion to CLs has been studied extensively[9–14] and shown to be influenced by the physicochemical surface properties of both the CLs and the bacteria, such as wettability and roughness. For instance, *Aeromonas hydrophilia* and *P. aeruginosa* adhere in a significantly higher amount to a more hydrophobic or water-repellent CL surface than to a hydrophilic hydrogel lens.[9]

Bacterial transmission from one substratum to another, as from a CL to corneal epithelial cells, has rarely been studied, but has been shown to be influenced by the physicochemical properties of all surfaces involved.[15,16] Significantly less *S. aureus*, for instance, migrated to finger pads from a cotton fabric than from a more hydrophobic cotton fabric.[15] In a recent study by Vermeltfoort et al.,[16] *P. aeruginosa* and *S. aureus* adhering to CLs were shown to be transmitted least to the more hydrophobic and rough lens types.

Bacterial adhesion to corneal epithelial cells has been studied in vitro and ex vivo and shown to be dependent on the bacterial strain and type of CL involved. Most studies on adhesion to epithelial cells involved *P. aeruginosa*, some strains of which have been described to be able to invade and kill the epithelial corneal cells.[17,18] Ren et al.[19] found that epithelial cells obtained from volunteers wearing high-oxygen-permeable lenses were less susceptible to bacterial adhesion than cells of volunteers wearing less permeable lenses.

For enhanced safety of CL use, the transmission of bacteria from the CL to the epithelial cells is an important topic to study, as it is one of the initial steps in the development of CL-related microbial keratitis. The purpose of this study was to quantify bacterial transmission from three different types of CLs to ex vivo porcine eyes, concentrating on the influence of surface roughness and hydrophobicity of the lens, to compare the bacteriologic safety of the lenses.

## METHODS

### Contact Lenses

In this study, three commercially available CLs were used, including a type of daily wear CL and two types of extended-wear lenses. The daily wear lens is made of etafilcon A, a homogeneous hydrogel, containing 58% water (Surevue; Vistakon, Johnson & Johnson, Jacksonville, FL) and belonging to FDA class IV (high water, ionic). The two extended-wear lenses were both made of a silicone hydrogel. One extended-wear lens used was made of lotrafilcon A, containing 24% water (Focus Night & Day; Ciba Vision, Atlanta, GA), and the other lens was made of balafilcon A, containing 36% of water (PureVision; Bausch & Lomb, Rochester, NY). Lotrafilcon A belongs to FDA class I (low water, nonionic), whereas balafilcon A is a low-water, ionic material belonging to FDA class III.

**TABLE 1.** Hydrophobicity by Water Contact Angles and Roughness of the Contact Lenses and Bacterial Strains

| Surface | $\theta_w$ (degrees) | $R_A$ (nm) |
|---|---|---|
| Lotrafilcon A | 34 ± 3 | 11.5 ± 1.5 |
| Balafilcon A | 59 ± 17* | 6.2 ± 0.8 |
| Etafilcon A | 34 ± 6 | 4.9 ± 1.2 |
| *S. aureus* 835 | 27 ± 4 | NA |
| *P. aeruginosa* 3 | 97 ± 13 | NA |
| Porcine eye | 0 | NA |

$n = 3$. NA, not applicable

* Water contact angles measured on one CL part were diverse, showing an overall decrease in angle during the series of measurements on one CL part.

## Bacterial Strains

Two different bacterial strains from patients with CL-related keratitis were used in this study. *S. aureus* 835, a hydrophilic strain,[10] was obtained from the Department of Medical Microbiology of University Hospital (Groningen, The Netherlands). A hydrophobic strain,[10] *P. aeruginosa* 3 was obtained by the courtesy of Donald G. Ahearn (Georgia State University, Atlanta, GA). From both strains, a frozen stock was precultured for 24 hours at 37°C in 10 mL tryptone soya broth (TSB; Oxoid, Basingstoke, UK). The preculture was used to inoculate a second culture (200 mL) for 18 hours at 37°C in ambient air in 250-mL Erlenmeyer flasks, to yield midexponential phase cells. *P. aeruginosa* 3 was harvested by centrifugation for 5 minutes at 9600*g*, *S. aureus* 835 by centrifugation for 5 minutes at 4000*g*. Both strains were washed twice with ultrapure water (Milli-Q Water Purification System; Millipore Corp., Bedford, MA) and resuspended in 10 mL ultrapure water. Bacteria were suspended to a density of $3 \times 10^8$ cells/mL in 0.9% saline supplemented with 2% (wt/vol) TSB to stimulate their metabolic activity and adhesion, while preventing their growth in suspension.[20]

## Porcine Eyes

Porcine eyes were obtained from recently killed pigs (Kroon BV, Groningen, The Netherlands). The eyes were chosen because their diameter is similar to that of human eyes,[21] allowing easy use of commercially available CLs. The pigs were destined for commercial use and were not specifically killed for the purpose of this study. Eyes were transported to the laboratory and were rinsed for 3 minutes with 200 mL demineralized water and 1 minute with 20 mL 0.9% saline. This procedure was shown to reduce the amount of bacteria on the cornea to an undetectable level when examined with confocal laser scanning microscopy (data not shown). During transmission experiments, eyes were stored at 21°C in a 100% humid environment to prevent dehydration. After the experiments, the corneas were examined for epithelial defects.

## Measurement of Surface Hydrophobicity and Roughness

The hydrophobicities of the CLs, porcine cornea, and bacterial cell surfaces were assessed by advancing water contact angle measurements, employing the sessile drop technique and a homemade contour monitor. On the CLs, water contact angles of 3-μL droplets were determined after placing the droplets on the concave sides. To prevent dehydration of the CL, measurements were recorded in air with 100% humidity, immediately after the lens was dipped five times in saline when it was removed from its container and after removal of excess fluid by gently tapping the CL on a tissue. Water contact angles of porcine corneas were measured similarly, whereas the measurement of contact angles on bacterial cell surfaces required special preparation, as described previously.[10] From each type of bacterial strain, CL or porcine cornea, three samples were analyzed, each with five water droplets for contact angle measurements.

The roughness of the CLs as used in the transmission experiments was assessed through atomic force microscopy (AFM; Nanoscope IIIa Dimersion 3100; Digital Instruments, Santa Barbara, CA). The microscope was operated in the contact mode, using a $Si_3N_4$ cantilever tip with a spring constant of 0.06 Newton-meters. Contact lenses with their concave sides up were put below the cantilever of the AFM to obtain height images in three dimensions at six places per sample. The AFM analysis was performed as fast as possible to minimize the dehydration of the CLs.
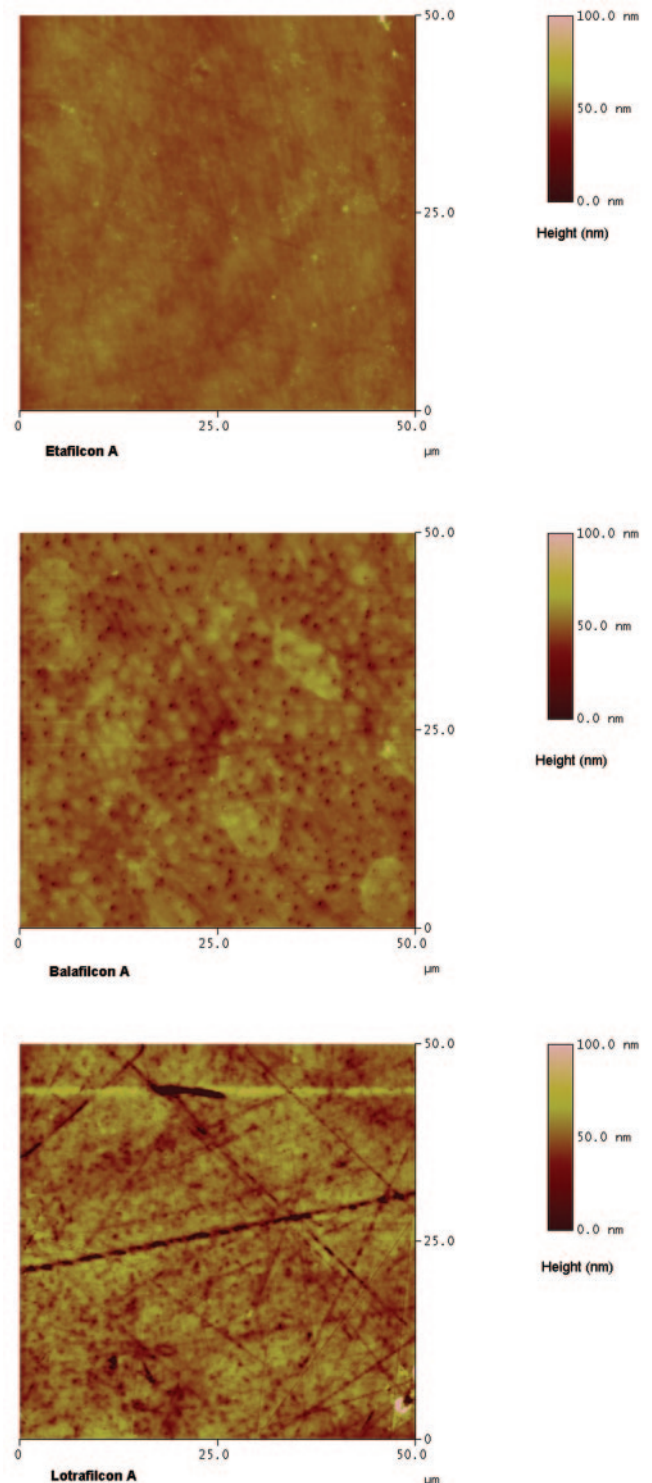


**FIGURE 1.** Examples of atomic force micrographs of the concave side of fully wetted contact lenses.
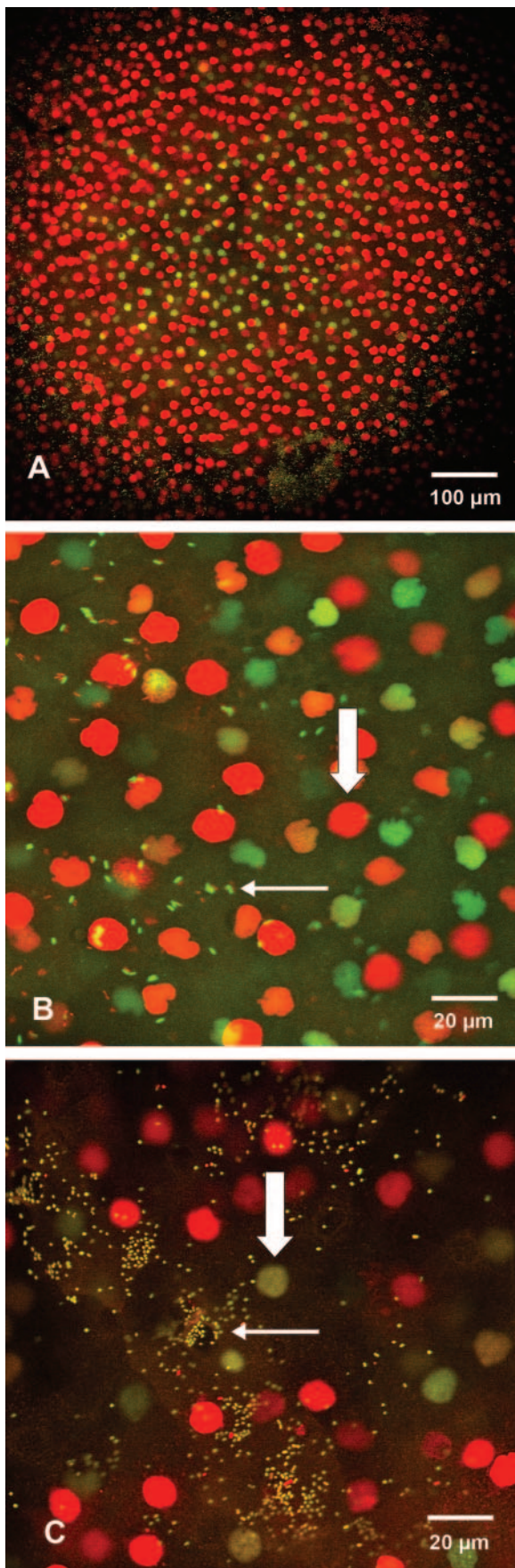
From each type of CL, three samples were used. The average roughness ($R_A$) was obtained from these images and indicates the average distance of the roughness profile to the center plane of the profile. The roughness of the porcine eye corneas were not measurable by AFM, because the viscosity of their surface.

## Bacterial Transmission

All CLs used in the transmission experiments were rinsed five times in 0.9% saline after removal from the lens storage package and put with their convex sides up in a well containing 5 mL of bacterial suspension in 0.9% saline supplemented with 2% (wt/vol) TSB. The CLs were incubated for 30 minutes with slight agitation on a rotating table. After incubation, the CLs were rinsed five times in sterile 0.9% saline and put on a clean porcine eye, wetted with 50 $\mu$L of 0.9% saline supplemented with 2% (wt/vol) TSB. During the experiments, the CLs and porcine eyes were stored at 21°C in 100% humidity. The lenses were removed from the eyes after 16 hours.

After separation and careful rinsing, the amount of bacteria adhering to the concave side of the CL and on the porcine corneas was determined in images made by confocal laser scanning microscopy (CLSM; TCS SP2; Leica, Heidelberg, Germany). Bacteria on eyes and CLs were stained with a stock solution of bacterial viability stain (live/dead *bac*light; Molecular Probes, Eugene, OR). Samples were excited with 488 and 543-nm light, yielding emitted light with wavelengths of 500 to 531 nm for viable bacteria (green) and 600 to 700 nm, for dead organisms (red). Serial scans were made of each surface at three randomly chosen positions on each sample. The scans covered a square of 187.5 $\mu$m$^2$ and had a resolution of 1024 $\times$ 1024 pixels per image. To determine the number of bacteria per square centimeter of lens surface, we generated on computer an overlay projection of all images made of one position. The percentage transmission of bacteria from the CL to the cornea was calculated by

$$\text{Transmission (\%)} = \frac{n_{\text{cornea}}}{n_{\text{cornea}} + n_{\text{CL}}} \times 100 \qquad (1)$$

in which $n_{\text{cornea}}$ and $n_{\text{CL}}$ are the number of bacteria adhering per square centimeter of the cornea or CL, respectively. Experiments were repeated six times with separately grown bacterial strains.

## Statistical Analysis

The data were analyzed with a univariate general linear model and Student's *t*-test, assuming equal variances (SPSS10 for Windows; SPSS Inc., Chicago, IL). The variable used in Student's *t*-test and the dependent variable used in the general linear model is a transformation of the percentage bacterial transmission. This transformation was performed to obtain a more normally distributed data set and was calculated according to

$$t = \log \frac{\text{Transmission}}{100 - \text{Transmission}} \qquad (2)$$

in which $t$ is the transformed transmission.

The independent variables "bacterial strain" and "type of CL" were tested for their correlation with the transformed transmission in a general linear model, using analysis of variance (ANOVA). The significance values obtained from ANOVA indicate the significance of the effect of an independent variable on the transmission. Significance values < 0.05 were considered to indicate significant effects.

**FIGURE 2.** Examples of confocal scanning laser micrographs of the porcine cornea after transmission experiments. (**A**) Overview micrograph of cornea, showing nuclei of epithelial cells and bacteria. (**B**) Micrograph of cornea showing epithelial cell nuclei (*large arrow*) and *P. aeruginosa* 3 (*small arrow*). (**C**) Micrograph of cornea showing epithelial cell nuclei (*large arrow*) and *S. aureus* 835 (*small arrow*).

**TABLE 2.** Transmission Percentages of *P. aeruginosa* 3 and *S. aureus* 835 from Different CLs to Porcine Corneas after 16 Hours

|  | *S. aureus* 835 | *P. aeruginosa* 3 |
| --- | --- | --- |
| Lotrafilcon A | 54 ± 19 | 51 ± 18 |
| Balafilcon A* | 82 ± 14† | 68 ± 16 |
| Etafilcon A* | 77 ± 10† | 60 ± 12 |

All data are averages of six experiments with separately grown bacterial strains.

\* Transmission percentages of *S. aureus* were significantly higher than of *P. aeruginosa.*

† Transmission percentage significantly higher than from lotrafilcon A.

## RESULTS

### Hydrophobicity and Roughness of Surfaces

The water contact angles of the bacterial cell surfaces and the CLs are compiled in Table 1. Whereas the lotrafilcon A and the etafilcon A lenses both seemed to be hydrophilic, the surface of the balafilcon A lens showed a higher water contact angle. Similarly, whereas *S. aureus* 835 had a hydrophilic surface, *P. aeruginosa* 3 appeared hydrophobic. The porcine cornea was fully wettable with water and thus had a 0° water contact angle in its hydrated state. Figure 1 presents AFM images of the CLs, and the average roughness of the CLs are summarized in Table 1. Lotrafilcon A lenses had the roughest surface, whereas the other lens types were both smoother (Table 1, Fig. 1).

### Transmission Experiments

Figure 2 shows examples of CLSM micrographs of the porcine cornea and a CL, made after 16 hours of transmission of *S. aureus* 835 or *P. aeruginosa* 3, and Table 2 shows the average transmission percentages of these bacteria from the three types of CLs to the porcine corneas. Transmission ranged from 54% to 82% for *S. aureus* 835 and from 51% to 68% for *P. aeruginosa* 3, depending on the CL considered. As can be seen in Table 2 Student's *t*-tests showed that there is significant difference between the two bacterial strains used for the balafilcon A ($P = 0.04$) and etafilcon A lens ($P = 0.006$), with higher percentages of transmission for *S. aureus* 835 than for *P. aeruginosa* 3. Furthermore, it can be seen in Table 2 that both strains were transmitted the least by the hydrophilic and rough lotrafilcon A lens compared with the other two lens types, though the transmission was only statistically significant for *S. aureus* ($P = 0.001$ and 0.006) and not for *P. aeruginosa* ($P = 0.098$ and 0.086). ANOVA results indicated that both bacterial strain ($P = 0.016$) and type of CL ($P = 0.003$) are influential factors in bacterial transmission from a CL to the cornea.

## DISCUSSION

In this study, a new model was used to evaluate the transmission of two bacterial strains from three types of CLs to ex vivo porcine corneas, without considering environmental influences, such as blinking and tear flow in the in vivo eye. *S. aureus* 835 showed greater transmission from balafilcon A and etafilcon A than *P. aeruginosa* 3, and both strains were transmitted least from a hydrophilic and rough contact lens type, notwithstanding that other strains may behave in a different way. At this point, it should be emphasized that transmission is but a single factor in the otherwise multifactorial process of the development of clinical microbial keratitis.

The transmission of bacteria from a CL to another substratum, like the cornea, requires detachment of bacteria from the "donating" CL and subsequent initial adhesion to the "receiving" substratum. The initial adhesion of bacteria is facilitated by the effect of all forces acting between the bacteria and substratum, such as Lifshitz-Van der Waals forces, electrostatic forces, and acid-based interactions. If the resultant force is attractive, initial reversible adhesion occurs and can become more irreversible with time.[22] The magnitude of the initial resultant force between a receiving substratum and a bacterium is often linked to the initial deposition rates of these bacteria on that substratum.[22] The strength of the adhesion force between the donating substratum and a bacterium is often considered to be related to the retention of bacteria after the application of a detachment force on the donating substratum.[22,23] Transmission is highest when both detachment of bacteria from the donating CL and initial adhesion to the receiving substratum are favorable.

Hydrophobicity[23] and roughness[12,22,24] of the donating CL has already been shown to influence bacterial retention and, in this study, was also suggested to influence bacterial transmission, which is clinically relevant for the choice of CLs for practical use. In our study, bacteria were transmitted least from the most hydrophilic and roughest CL, which may relate to a high retention of adherent bacteria. From a thermodynamic point of view, the hydrophilic *S. aureus* 835 can indeed be expected to be transmitted least from a hydrophilic than from a hydrophobic CL surface, as it favors hydrophilic surfaces above more hydrophobic interfaces.[22] However, hydrophobic *P. aeruginosa* 3 was also transmitted in lowest numbers (although not statistically significant) from the hydrophilic lens, possibly because of the presence of cell surface appendages such as fibrils and fimbriae, facilitating extra–short-range interactions with the CL surface.[25] In vivo and in vitro studies have shown that rougher surfaces have more bacterial retention after the application of detachment forces, indicating higher adhesive strength between bacteria and a rougher donating substratum.[12,22,24] The rougher lotrafilcon A CL may present more surface area that facilitates specific binding with bacterial cell surface molecules,[24,25] which consequently would lead to the lower transmission found in the present study for this CL type compared with the other two smoother lens types.

*S. aureus* was found to have higher transmission percentages compared with *P. aeruginosa* for etafilcon A and balafilcon A lenses. The hydrophilic *S. aureus* 835 probably has an higher affinity for the hydrophilic porcine cornea than the more hydrophobic *P. aeruginosa* 3, causing its greater transmission.[22] The higher transmission percentages of *S. aureus* are not in line with clinical findings, in which *P. aeruginosa* is more often found to be the causative organism of CL-related microbial keratitis, which could be because this study neglected possible invasive trading of the bacterial strains.[4,7] The *P. aeruginosa* strain used in this study is marked as invasive,[26,27] which means these bacteria invade the epithelial cell membrane, leading to an higher risk of microbial keratitis. In this study, *P. aeruginosa* 3 was found to invade and colonize the epithelial cell layer, with a slight preference for artificial damage to the corneal epithelium, confirming the increased risk of keratitis resulting from corneal damage in clinic.[28]

This study describes an ex vivo model for determining bacterial transmission from contaminated CLs to ex vivo porcine corneas. *S. aureus* 835 gave higher transmission percentages than *P. aeruginosa* 3, whereas both strains were transmitted least from a hydrophilic and rough CL. These results show the importance of the physicochemical surface properties of CLs in bacterial transmission to the cornea.

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