

ORIGINAL ARTICLE

The Effect of Octylglucoside and Sodium Cholate in *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* Adhesion to Soft Contact Lenses

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ABSTRACT

Purpose. In this study, the effect of the natural surfactants octylglucoside and sodium cholate in inhibiting *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* adhesion to conventional and silicone-hydrogel contact lenses (CL) was assessed. Hydrophobicity was also evaluated to conditioned and nonconditioned CL.

Methods. The inhibiting effect of the tested surfactants was determined through “in vitro” adhesion studies to conditioned and nonconditioned CL followed by image acquisition and cell enumeration. Hydrophobicity was evaluated through contact angle measurements using the advancing type technique on air.

Results. Sodium cholate exhibits a very low capability to inhibit microbial adhesion. Conversely, octylglucoside effectively inhibited microbial adhesion in both types of lenses. This surfactant exhibited an even greater performance than a multipurpose lens care solution used as control. Octylglucoside was the only tested surfactant able to lower the hydrophobicity of all CL, which can explain its high performance.

Conclusions. The results obtained in this study point out the potential of octylglucoside as a conditioning agent to prevent microbial colonization.

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Key Words: octylglucoside, sodium cholate, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, inhibition of adhesion

Over the last few decades the number of contact lens (CL) wearers has grown rapidly because of the esthetic, therapeutic, visual, and comfort reasons. There are several kinds of lenses commercially available. However, soft CL are the most common. These lenses are composed of hydrophilic monomers such as hydroxyethylmethacrylate, *N*-vinyl pyrrolidone, methacrylic acid, and polyvinyl alcohol.^{1,2} Recently, the introduction of silicone-containing hydrogel CL having the same comfort and significantly higher oxygen permeability than conventional-hydrogel has resulted in a new generation of soft CL. The high oxygen permeability, on account of the siloxane component, makes it possible to wear these lenses on a continuous basis for 30 days.^{1–3}

The occurrence of CL associated keratitis as well as other ocular complications has been a target of continuous research in several fields. When a CL is placed in the eye, the lachrymal tear components are adsorbed on its surface, building an organic substrate for subsequent microbial adhesion.⁴ In particular, when the corneal tissues are no longer intact due to hypoxic conditions or mechanical friction, microbes can invade the cornea and induce an ocular infection.^{4,5} So, the development of strategies such as the improvement of lens materials and lens care systems that avoid or decrease CL associated infections are very important aspects of soft CL research. The incorporation of surfactants in the lens care systems is useful not only to solubilize the organic tear film components

TABLE 1.
Contact lenses and their properties

Category	Material	Commercial name	Manufacturer	FDA group	Charge	Water content (%)	Surface treatment
Conventional hydrogel	Nelfilcon A	Focus Dailies	CIBA Vision	II	Nonionic	69.0	No
	Etafilcon A	Acuvue	Johnson and Johnson Visioncare	IV	Ionic	58.0	No
Silicone hydrogel	Lotrafilcon B	O ₂ Optix	CIBA Vision	I	Nonionic	33.0	25 nm plasma coating with high refractive index
	Balafilcon A	Purevision	Bausch & Lomb	III	Ionic	36.0	Plasma oxidation producing glassy islands

adsorbed on lens surface, but also to disrupt microbial membranes.⁶ Nonetheless, surfactants are also able to modify the CL surface properties and thus may inhibit microbial adhesion.^{7,8}

Octylglucoside is a nonionic and nontoxic surfactant which belongs to the alkylglucoside class,⁹ being frequently used to solubilize membrane bound proteins in their native state. Sodium cholate is a negatively charged (anionic) and nontoxic surfactant that belongs to the bile salts class. The use of sodium cholate has already been tested and when used below 0.5% (w/v)¹⁰ is harmless to the ocular tissues. The aim of this work is to compare the effect of two natural surfactants, octylglucoside and sodium cholate and one commercial multipurpose lens care solution which incorporates the surfactant poloxamine in inhibiting the adhesion of one strain of *Staphylococcus epidermidis* and one of *Pseudomonas aeruginosa* to conventional hydrogel and silicone-hydrogel CL. These bacterial species are two of the most frequent pathogens^{4,5,11} involved in the occurrence of microbial keratitis and thus considered representative for this study. The efficacy of the surfactants was tested on CL belonging to each FDA group.

MATERIALS AND METHODS

Contact Lenses

CL from each of the four FDA groups were used in this study. Group I materials are nonionic and possess a water content lower than 50%. Group II materials have water content of 50% or greater and are nonionic. Group III lenses are made of low-water content ionic materials and, finally, group IV lenses consist of high-water content ionic materials. The properties and commercial designations of the lenses used in this study are detailed in Table 1.

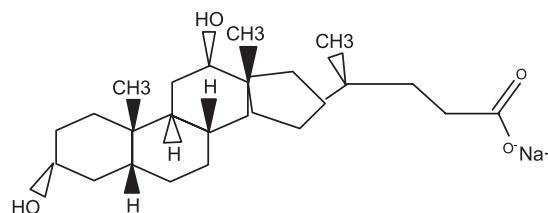
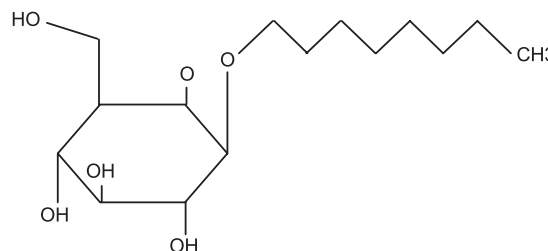
Surfactants and Multipurpose Solutions

The tested surfactants were n-octylglucoside [n-octyl-β-D-glucopyranoside] (Sigma Aldrich, Germany), a nonionic surfactant, and sodium cholate (Sigma Aldrich, Germany), an anionic surfactant. The physical-chemical properties of these surfactants are detailed in Table 2 and their structures represented in Figures 1 and 2. The concentration used for both was half of their respective critical micelle concentration. Surfactant solutions were prepared with sterile deionized water and used immediately after preparation. The multipurpose lens care solution was Renu Multiplus

TABLE 2.
Properties of octylglucoside and sodium cholate

Surfactant	CMC (% w/v)	Molecular weight (g/mol)	Chemical formula
Octylglucoside	0.60	292.38	C ₁₄ H ₂₈ O ₆
Sodium cholate	0.73	430.53	C ₂₄ H ₃₉ O ₅ Na

CMC, critical micelle concentration.

**FIGURE 1.**
Schematic representation of the sodium cholate molecule.**FIGURE 2.**
Schematic representation of the octylglucoside molecule.

with Hydranate (Bausch & Lomb, US). This solution is composed of 1% poloxamine (nonionic surfactant), 0.0001% Dymed (cationic biocide), 0.03% Hydranate (protein remover), and ethylene diamine tetraacetic acid (EDTA).

Bacterial Strains and Growth Conditions

The strains used in this study were the clinical isolate *S. epidermidis* 9142, and *P. aeruginosa* ATCC 10,145 (ATCC, American Type Collection Culture). *S. epidermidis* 9142 is a well-known producer of the major surface polysaccharide promoting coagulase negative staphylococci adherence and biofilm formation, referred

to as either polysaccharide intercellular adhesin or by its chemical composition, poly-*N*-acetyl glucosamine. This strain was kindly provided by Gerald B. Pier, Harvard Medical School, US, and its adhesion and biofilm formation capabilities were characterized in previous studies.¹² *P. aeruginosa* ATCC 10,145 was obtained from the ATCC and was isolated by F. Kavanagh (Merck Sharp and Dohme).

A 4°C culture stock was inoculated into an Erlenmeyer flask containing 10 mL of tryptic soy broth (TSB, Merck, Germany) and incubated for 24 h at 37°C. After this period, 1 mL of the culture suspension was transferred to a second Erlenmeyer flask containing 30 mL of TSB and incubated for 18 h at 37°C in order to obtain a midexponential growth culture. Cells were harvested by centrifugation (15 min, 5000*g*) and washed twice with Millipore water. Finally, the cells were resuspended in phosphate buffered saline (PBS: 8 g L⁻¹ NaCl, 0.2 g L⁻¹ KCl, 0.2 g L⁻¹ KH₂PO₄, 1.15 g L⁻¹ Na₂HPO₄) and the concentration adjusted to 1 × 10¹⁰ CFU/mL. Before the experiments, cell viability was evaluated through plating and colony forming units enumeration. The results showed that both strains maintain their viability after 18 h of incubation (data not shown).

Contact Angle Measurements

The CL relative hydrophobicity was determined through contact angle measurement with Millipore water using the advancing contact angle as described by Bruinsma et al.⁴ Contact angles were measured on nonconditioned and conditioned CL using the apparatus OCA 20 (Dataphysics). The conditioning process was performed by simple immersion of the lenses in the surfactant solution, or in the multipurpose lens care solution for a 16-h period. For the measurements, lenses were cut into four pieces and placed on a microscope slide. The excess of moisture was removed by gentle blotting with absorbent paper. These measurements were repeated 15 times per contact lens material at room temperature (22°) and a humidity of 50% ± 3%.

Adhesion Assays and Image Acquisition

The method used to assess bacterial adhesion to CL was the static adhesion assay. Each CL was immersed in a well of a 24-well microtiter plate containing 1 mL of a cell suspension (6 × 10¹⁰ CFU/mL) prepared in PBS. The tissue microtiter plate was then incubated for 2 h at 37°C and after this period each CL was removed and washed three times by immersing the lens in clean sterile PBS solution for 10 s. This washing step was carefully performed to remove only the cells that were suspended in the liquid interface formed along the lens surface and to minimize adhered cells detachment as described by Cerca et al.¹³ The adhesion assays were performed with non-conditioned (control) and conditioned CL. The lenses were conditioned by simple immersion in the surfactant solutions, or in the multipurpose lens care solution for 16 h followed by the adhesion assay. The adhesion assays were made in triplicate and repeated twice for each CL type and each conditioning agent.

After the adhesion assays, two opposite edges of each CL were cut to flatten the surface and the lens mounted on a microscope slide. Cell quantification was performed using a phase contrast

microscope (Carl Zeiss, Germany) coupled to a 3 CCD video camera (Carl Zeiss) that acquires images at a magnification of 1622× with a resolution of 1300 × 1030 pixels and 20 images were randomly take from each CL. To eliminate image interferences, the background was captured and subtracted from the original image. Cells were enumerated using the Sigma Scan Pro program and for the magnification used 1 cm² was equivalent to 3906.25 captured images.

The % of adhesion inhibition by each solution was calculated as follows:

% Inhibition

$$= \left(\frac{\# \text{cells adhered to non-conditioned CL} - \# \text{cells adhered to conditioned CL}}{\# \text{cells adhered to non-conditioned CL}} \right) \times 100$$

Statistical Analysis

Data analysis was performed using the statistical program, SPSS (Statistical Package for the Social Sciences). After the evaluation of data distribution by K-test, contact angles data were compared using the parametric test analysis of variance (ANOVA) with Tukey's pairwise comparison whereas the extent of adhesion was compared by the nonparametric Mann–Whitney U test. All tests were performed with a confidence level of 95%.

RESULTS

Contact Angles

Figure 3 presents the water contact angle values of the studied CL. According to van Oss and Giese,¹⁴ a surface can be considered hydrophobic if the water contact angle exceeds 50° and hydrophilic if it is inferior to 50°. Thus, contact angles of nonconditioned CL showed that silicone hydrogel CL are hydrophobic, whereas the conventional hydrogel CL are hydrophilic with Nelfilcon A being the most hydrophilic one (Etafilcon A, *p* = 0.023; Balafilcon A, *p* = 0.000; Lotrafilcon B, *p* = 0.000).

After conditioning the CL with the surfactants or the multipurpose solution, the contact angles generally decreased except for

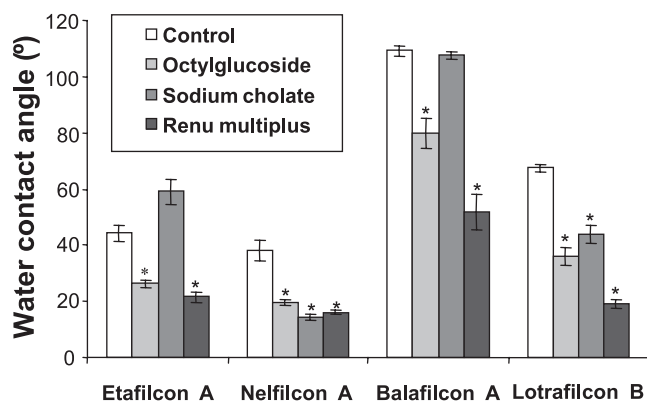


FIGURE 3.

Water contact angles of non-conditioned and conditioned CL measured at room temperature (one-way ANOVA, Tukey's with 95% confidence level). Error bars represent standard deviations.

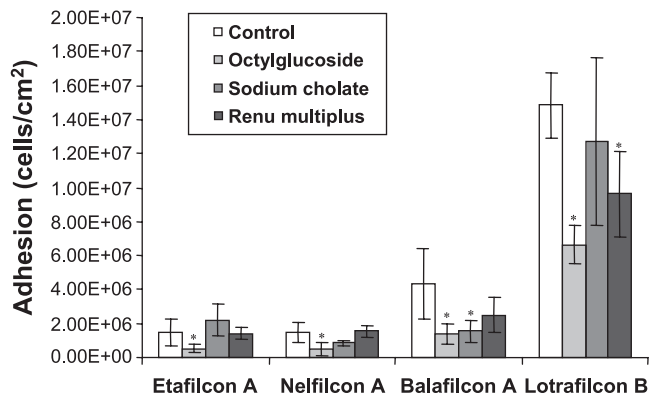


FIGURE 4.

Number of cells of *Staphylococcus epidermidis* adhered to nonconditioned and conditioned CL with octylglucoside, sodium cholate, and the multipurpose lens care solution. *Statistically superior compared to the control (Mann–Whitney U Test). Error bars represent standard deviations.

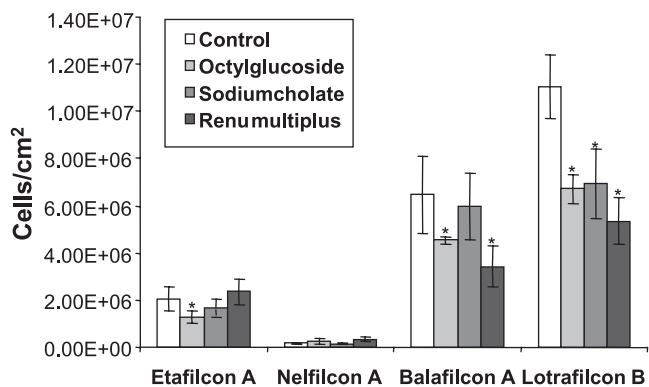


FIGURE 5.

Number of cells of *Pseudomonas aeruginosa* adhered to nonconditioned and conditioned CL with octylglucoside, sodium cholate, and the multipurpose lens care solution. *Statistically different compared to the control (Mann–Whitney U Test). Error bars represent standard deviations.

Balafilcon A ($p = 1.00$) and Etafilcon A conditioned with sodium cholate, which increased ($p = 0.000$). This result indicates that sodium cholate is not such an effective surface agent as octylglucoside or the multipurpose solution.

Bacterial Adhesion to Nonconditioned CL

Static adhesion results can be observed in Figures 4 and 5. For both tested strains it was observed that bacterial adhesion occurred

TABLE 3.

Inhibition of adhesion (average values) promoted by octylglucoside, sodium cholate, and the multipurpose solution (% \pm standard deviation)

	Etafilcon A	Nelfilcon A	Balafilcon A	Lotrafilcon B
<i>Staphylococcus epidermidis</i> 9142				
Octylglucoside	65.5 \pm 24.4	68.0 \pm 8.6	68.2 \pm 23.7	55.3 \pm 3.0
Sodium cholate	-45.6 \pm 3.2	42.8 \pm 11.7	64.7 \pm 21.8	14.5 \pm 3.0
Multipurpose solution	5.6 \pm 1.8	-2.8 \pm 0.5	42.2 \pm 10.2	35.7 \pm 1.5
<i>Pseudomonas aeruginosa</i> ATCC 10145				
Octylglucoside	37.6 \pm 4.7	-63.6 \pm 32.7	30 \pm 6.6	39.0 \pm 2.6
Sodium cholate	20.3 \pm 1.3	4.6 \pm 0.65	7.8 \pm 0.26	37.0 \pm 0.5
Multipurpose solution	-14.1 \pm 0.6	-96.6 \pm 33.7	47.0 \pm 2.8	51.5 \pm 0.08

in larger extent to silicone hydrogel CL than to conventional hydrogel CL. Adhesion of *S. epidermidis* 9142 and *P. aeruginosa* ATCC 10,145 to Balafilcon A and Lotrafilcon B was significantly greater than that observed in Etafilcon A and Nelfilcon A ($p < 0.05$).

Adhesion to Conditioned CL and Inhibition of Adhesion

Figures 4 and 5 and Table 3 present, respectively, the results of microbial adhesion to conditioned CL and the % of inhibition promoted by the surfactant solutions and the multipurpose solution. Generally, octylglucoside exhibited the best performance for both strains and the tested CL. Octylglucoside was very effective in inhibiting *S. epidermidis* adhesion, because all CL conditioned with this surfactant showed a significant decrease in the number of adhered cells (Etafilcon A, $p = 0.021$; Nelfilcon A, $p = 0.021$; Balafilcon A, $p = 0.020$; and Lotrafilcon B, $p = 0.009$). This surfactant was also effective against *P. aeruginosa* in all lenses (Etafilcon A, $p = 0.008$; Balafilcon A, $p = 0.020$; and Lotrafilcon B, $p = 0.020$) except for Nelfilcon A, probably because *P. aeruginosa* showed very low levels of adhesion to this material. Concerning sodium cholate, this surfactant only inhibited the adhesion of the *S. epidermidis* strain to Balafilcon A CL ($p = 0.021$) and of *P. aeruginosa* to Lotrafilcon B. The multipurpose solution, did not demonstrated a significant inhibition effect in *S. epidermidis* adhesion with the exception of Lotrafilcon B, whereas for *P. aeruginosa* this effect was relevant in Balafilcon A ($p = 0.006$) and Lotrafilcon B ($p = 0.014$).

DISCUSSION

In this work, the preconditioning effect of two surfactants and one multipurpose solution on bacterial adhesion to CL was evaluated. The adhesion assays were performed on unworn CL, however, it must be considered that in situ the CL become rapidly conditioned with adsorbed components of the tear film such as proteins and lipids,^{15–17} which may influence lens surface properties and thus microbial adhesion. Nevertheless, this fact does not invalidate the methodology used, because the purpose of this work is to study the CL preconditioning as a way to promote the inhibition of adhesion and as a palliative strategy to avoid ocular complications. The modification in CL surface hydrophobicity due to surfactant conditioning was also evaluated.

Contact angle measurements (Fig. 3) revealed that the silicone hydrogel CL, Balafilcon A and Lotrafilcon B are hydrophobic, whereas conventional hydrogel CL are hydrophilic. Silicone-hydrogel CL hydrophobicity was already demonstrated and explained by the presence of silicone in the lens matrix which is a hydrophobic monomer. Contact angles have also revealed that the surfactants and the multipurpose solutions are capable of modifying the CL surface properties. Generally, conditioned CL resulted in a decrease of the water contact angle with the exception of Balafilcon A and Etafilcon A with sodium cholate. It is commonly accepted that surfactant adsorption depends mainly on the surfactant structure and surfactants with longest alkyl chain usually adsorb the most.⁹ Sodium cholate exhibits a very different chemical structure from octylglucoside and poloxamine which may explain its lower performance. In fact, this bile salt is a planar molecule, (Fig. 1) punctuated with hydrophilic groups conversely to octylglucoside or poloxamine, which exhibit well defined hydrophilic and hydrophobic domains. The adsorption of octylglucoside and poloxamine on the lens surface through the hydrophobic moieties, exposing the hydrophilic groups to the aqueous media certainly contributed to the decrease of the hydrophobicity of all CL.

Regarding bacterial adhesion to nonconditioned lenses, silicone-hydrogel CL revealed to be more prone to *S. epidermidis* and *P. aeruginosa* adhesion than conventional hydrogel CL. These results seem to be strongly related with the lens surface hydrophobicity. In a previous study we have demonstrated that silicone-hydrogel CL are more prone to *S. epidermidis* and *P. aeruginosa* adhesion than conventional hydrogel,¹⁸ corroborating other “in vitro” studies using different microorganisms.^{19–21}

Concerning the conditioning effect of the surfactants or the multipurpose solution, this study showed that the modification of the surface properties, particularly the decrease of hydrophobicity not always leads to a decrease in bacterial adhesion. It is well established that surfactants are able to modify the surface properties of materials and thus influence adhesion,^{22–24} however in this study only CL conditioned with octylglucoside, revealed a significant decrease of hydrophobicity as well as a reduction in the extent of microbial adhesion compared with the control (Figs. 4 and 5, and Table 3). This result is most probably related with the amphiphilic properties of the surfactant molecules as well as their structure. Accordingly, well-defined hydrophilic/hydrophobic regions of both octylglucoside and poloxamine enabled them to coat the lens in a uniform and consistent way, in opposite to sodium cholate. Sodium cholate only inhibited microbial adhesion in Balafilcon A, although it did not reduce the lens hydrophobicity. This CL has a nonuniform surface, presenting “silicate islands” and probably sodium cholate molecules were adsorbed between these “islands” building a physical barrier against bacterial adhesion. The multipurpose solution was effective in inhibiting the adhesion of the *S. epidermidis* strain to Lotrafilcon B and the *P. aeruginosa* strain to Balafilcon A and Lotrafilcon B. A better performance of this solution was expected since it incorporates the surfactant poloxamine which possesses antimicrobial properties^{25,26} and in addition, has a higher surfactant concentration than the tested surfactant solutions. Nevertheless, the presence of other complex components in the multipurpose solution may have contributed for lowering its performance.

This study provides evidence that octylglucoside can effectively inhibit bacterial adhesion either to conventional or to silicone-

hydrogel CL. This finding is most likely related to their amphiphilic properties as their molecular structure. Many other conditioning agents such as poly(ethylene glycol),²⁷ salicylate²⁸ and heparin²⁹ have been tested on CL with the aim of reducing microbial adhesion. Still, octylglucoside has the increased advantage of inhibiting adhesion and being nontoxic and inexpensive. Despite the good results obtained for octylglucoside more experiments must be performed to test if the inhibiting capability of octylglucoside is affected by other chemical components that may be present in multipurpose solution such as biocides and preservatives.

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