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The influence of lens material and lens wear on the removal and viability of *Staphylococcus epidermidis*

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Abstract

Purpose: The aim of this study was to evaluate the influence of lens material and lens wear on the removal capability of *Staphylococcus epidermidis*. Assessment of viability of remaining adhered bacteria was another goal of this work. Four silicone hydrogel materials (galyfilcon A, balafilcon A, lotrafilcon A, lotrafilcon B) and one conventional hydrogel material (etafilcon A) were assayed.
Methods: Detachment studies on *S. epidermidis* were carried out in a parallel plate flow chamber. Contact lenses (CLs) were fitted to the bottom of the flow chamber and a bacterial suspension was perfused into the system, promoting bacterial adhesion. Afterwards, detachment was stimulated using a multipurpose solution (MPS, ReNu Multiplus®) and the percentage of removed bacteria estimated through microscopic observation and enumeration. Remaining adhered bacteria were stained with propidium iodide (PI) and enumerated in order to assess their viability. Additionally, the worn lenses were observed by confocal laser scanning microscopy (CLSM) to visualize bacterial distribution along the lens surfaces.
Results: Bacterial removal was significant ($p < 0.05$) for both unworn and worn galyfilcon A and etafilcon A. Galyfilcon A exhibited a detachment percentage of 59.1 and 63.5 while etafilcon A of 62.6 and 69.3, both for unworn and worn lenses, respectively. As far as bacterial viability is concerned, it was found that worn lenses exhibit a superior amount of non-viable bacteria than unworn CLs. Images obtained by CLSM revealed an irregular bacterial distribution for all lens materials.
Conclusions: It appears that surface and/or bulk structure of the lens material affects removal of *S. epidermidis* while CL wear influences their viability.

Keywords: Bacterial detachment; Silicone hydrogel; Cell viability

1. Introduction

CL solutions were first produced in the late 40s and have been developed ever since. These solutions should comprise several functions as to enhance CL wettability, prevent the build-up of deposits and provide effective disinfection against pathogenic microorganisms [1]. Currently, MPS are the most popular CL solutions since they permit in a single step to clean, rinse and disinfect [2]. Disinfection is mainly promoted by the presence of biocides and it is essential to prevent ocular infections, which ultimately can lead to vision impairment. This process may be affected by numerous factors, which include the biocide, the challenging microbe, the material and the presence of organic matter [3–6]. Due to the presence of surfactants, MPS may also promote bacterial removal; however, according to a previous study it was not significant [7].

Polyhexamethylene biguanide (PHMB) is one of the most popular biocide agents and has been used since the mid 70s in ophthalmic solutions. It is a polymeric cationic surfactant that belongs to the biguanide family and is

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currently used in several commercially available MPS. PHMB performs by enabling membrane disruption and lysis in bacteria, which results in their death [8,9].

Several studies have investigated disinfection and bacterial detachment from CLs [7,10–17]. However, since silicone hydrogel CLs were launched, very few works have been performed with this type of material. The present study aimed to evaluate the influence of lens material and lens wear on the detachment capability of *Staphylococcus epidermidis*. Four silicone hydrogel materials (galyfilcon, balafilcon A, lotrafilcon A, lotrafilcon B) and one HEMA material (etafilcon A) were worn daily, for one month with nightly disinfection with a single multipurpose solution (ReNu Multiplus). In addition, viability and distribution of the remaining adhered bacteria were analysed through epifluorescence microscopy and CLSM, respectively. Matched unworn lenses served as the control.

2. Materials and methods

2.1. Contact lenses

The CLs used in this study are detailed in Table 1.

2.2. Clinical trial

Thirty-one subjects from both genders enrolled the present study, excluding any lost to follow up. The volunteers were predominantly from the north of Portugal and the average age was 23.6 ± 5.5 years. These were chosen according to the following parameters: they have never worn CLs before (neophytes), they were not taking any medications during the trial, they did not suffer from any kind of ocular allergy and they had no tendency for dry eye syndrome.

Subjects were divided into four groups. Eight individuals were fitted with galyfilcon A, eight with balafilcon A, eight with lotrafilcon A and seven with lotrafilcon B. Etafilcon A was used as contralateral pair into the four groups since a parallel study was ongoing in order to evaluate morphological changes between one eye fitted with a silicone hydrogel and the other fitted with a conventional hydrogel. According to the manufactures' recommendations, one of the silicone hydrogel lenses, galyfilcon A wear is recommended under a two-week planned replacement modality. However, in this study, it was worn for 30 days in order to establish a comparison with the other silicone hydrogel lenses. None of the groups was aware of the CL material or brand they were using.

Silicone hydrogel CLs were used during 30 days and etafilcon A for 15 days (replaced at the end of 15 days), according to a daily wear schedule. The subjects were instructed to remove their lenses and place them directly into a solution (ReNu MultiPlus®, Bausch & Lomb, Inc. polyhexanide 0.0001%, hydranate 0.03% and poloxamine 1%) for overnight disinfection, between 12 and 14 h wear (no rub or rinse). At the end of the wearing period, each lens was aseptically removed from the eye and placed in a sterile vial containing a sterile saline solution (0.9% NaCl). Vials were labelled with a code and details of the lens material. The CLs were stored at 4 °C no longer than 5 days until analysis. Unworn CLs were stored at room temperature (20 °C ± 2) and managed under sterile conditions until the beginning of experiments.

Each subject signed an informed consent following an explanation related to the nature of the study and its possible risks to the participant. No significant adverse events occurred throughout the course of this study.

2.3. Microorganism and growth conditions

The challenging microorganism was *S. epidermidis* 9142. This Gram-positive bacterium is a clinical isolate and was kindly provided by Dr. Gerald B. Pier, Harvard Medical School, Boston, USA. Its adhesion and biofilm formation capabilities were characterised in a previous study [18].

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 mid-exponential growth culture. Cells were harvested by centrifugation (15 min, 4000 rpm) and washed twice with ultrapure water. Finally, the cells were resuspended in phosphate buffer saline (PBS, 8 g l^{-1} NaCl 0.2 g l^{-1} KCl 0.2 g l^{-1} KH_2PO_4 1.15 g l^{-1} Na_2HPO_4 pH 7.4) and the concentration adjusted to 6×10^{10} CFU/ml.

Table 1
Contact lenses properties

Commercial name	Manufacturer	Material	FDA group	Water content (%)	Surface treatment
Acuvue®	Johnson & Johnson Vision Care	Etafilcon A	IV	58	No
Acuvue® Advance™	Johnson & Johnson Vision Care	Galyfilcon	I	47	No
Purevision™	Bausch & Lomb, Inc.	Balafilcon A	III	36	Plasma oxidation
Focus® Night & Day™	CIBA Vision	Lotrafilcon A	I	24	25 nm plasma coating with high refractive index
O_2Optix™	CIBA Vision	Lotrafilcon B	I	33	25 nm plasma coating with high refractive index