

Bacterial adhesion to Conventional and Silicone hydrogel contact lenses

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Abstract

Purpose: The aim of this study was to investigate the adhesion of bacteria to worn silicone hydrogel and conventional soft contact lenses.

Methods: Bacterial adhesion experiments / assays were performed on 24 worn and 6 unworn soft contact lenses each of different materials (high- and low- gas permeable lenses) using the strains such as *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

Results: *P. aeruginosa* adhered in increased number to worn than unworn Lotrafilcon A and conventional lenses. However, a higher number of *P. aeruginosa* adhered to unworn than worn Lotrafilcon B, the difference in the mean adhesion was not significant ($p = 0.66$). *S. aureus* adhered in significantly decreased number to worn Lotrafilcon A, nelfilcon A, nesofilcon A, etafilcon A and omafilcon A ($p < 0.05$); but significantly higher number adhered to worn than unworn polymacon ($p < 0.05$). Lens wear had no effect on the adhesion of *S. aureus* to Lotrafilcon B ($p > 0.05$). The least adhesion of *P. aeruginosa* to worn contact lenses was seen with polymacon, while *S. aureus* adhered in least number to worn Lotrafilcon A compared to the other contact lens materials that demonstrated the same trend in adhesion.

Conclusion: The higher adhesion of *P. aeruginosa* to worn lenses is consistent with the claim that it is the most implicated in all culture-positive contact lens related bacterial keratitis. Lens wear has different effects on bacterial adhesion, which may be due to type of lens materials and bacterial species/genera studied.

Keywords: Silicone hydrogel lenses, conventional lenses, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, bacterial adhesion.

Introduction

Contact lenses are medical devices made of biomaterials and used as alternative to spectacle lenses for correction of refractive ametropia,

presbyopia and other purposes like cosmesis and ocular therapeutics. The wear of contact lens has remained a risk factor for the development of various adverse effects such as microbial keratitis,¹

1. Green M, Apel A, Stepleton, F. Risk factors and causative organisms in microbial keratitis.

contact lens-related acute red eye,² contact lens induced peripheral ulcer³ and infiltrative keratitis.⁴ The adhesion and colonization of contact lenses by microbes particularly bacteria,¹ have been implicated as a major factor in the initiation of adverse effects.⁵ Lens deposits or defects, changes in pH as well as oxygen and carbon dioxide concentration, hypoxia, cytotoxicity of care solutions, and corneal surface disruption are the other causes of inflammation, which may be present alone or in combination with the microbial load on the lens. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the predominant microorganisms implicated in contact lens-related microbial adverse events.^{1,6} Microorganisms like *Serratia marcescens*,² Coagulase-negative *Staphylococci* (CNS),¹ fungus⁷ and *Acanthamoeba* sp⁸ are less frequently involved. in microbial infection, although they have been implicated at one point or the other. *P. aeruginosa* has been linked with sight-threatening microbial keratitis, which remains the most severe complication of contact lens wear.^{9,10} *Pseudomonas aeruginosa* has been considered the predominant causative agent that has the capacity to induce microbial keratitis (MK) which accounts for 40 to 70% of MK cases worldwide.¹¹ *Pseudomonas aeruginosa* and

Staphylococcus aureus together are responsible for about 50% of total culture positive contact lens-related microbial keratitis.^{1,12} Difference in data on bacterial adhesion to lenses between studies has been attributed to a range of assay conditions such as strains/types of bacteria, contact lens types, inoculum sizes, the nutritional content of media and the incubation time for adhesion to occur.¹³ Dutta and Willcox⁵ used two soft lens materials (etafilcon A and the silicone hydrogel senofilcon A) in their bacterial adhesion study while Vijay et al¹⁴ used silicone hydrogel lenses of different materials. Studies have shown that the multipurpose solution bottles can easily be contaminated and become a source of microbial contaminant for the lens storage cases, adherence to the lens, and cause of inflammatory reaction/infection of the cornea.^{15,16} Microbial adhesion to worn contact lenses is fundamental to the initiation of cascade of events characterizing the pathogenicity of infections of the cornea by microorganisms termed microbial keratitis.

Once any biomaterial like contact lens is exposed, bacterial colonization occurs because of the engagement of bacterial adhesins on their surface with biomaterial surface.^{17,18} Accumulation of bacterial cells on contact lens surfaces has been

2. Holden BA, La Hood D, Grant T, Newton-Howes J, Baleriola-Lucas C, Willcox MD, Sweeney DF. Gram-negative bacteria can induce contact lens related acute red eye (CLARE) responses. *Contact Lens Association of Ophthalmology Journal* 1996; 22: 47 - 52.
3. Wu P, Stapleton F, Willcox MD. The causes of and cures for contact lens-induced peripheral ulcer. *Eye Contact Lens* 2003; 29: S63 - S66.
4. Willcox M, Sharma S, Naduvilath TJ, Sankaridurg PR, Gopinathan U, Holden BA. External Ocular surface and Lens microbiota in contact lens wearers with corneal infiltrates during extended wear of hydrogel lenses. *Eye Contact Lens* 2011; 37: 90 - 95.
5. Dutta D, Willcox, MDP. A laboratory Assessment of factors That Affect Bacterial Adhesion to contact lenses. *Biology* 2013; 2(4): 1268 - 1281.
6. Otri AM, Fares U, Al-qaba MA, Midi A, Farah LA, Saril DG, Maharajam S, Dua HS. Profile of sights threatening infectious keratitis: A prospective study. *Acts Ophthalmologica* 2012; 23: 76 - 81.
7. Tuli SS, Iyer SA, Driebe WT Jr. Fungal keratitis and contact lenses: An old enemy unrecognized or a new nemesis on the block? *Eye Contact Lens* 2007; 33: 415 - 417.
8. Yoder JS, Verani J, Heidman N, Hoppe-Bauer J, Alfonso EC, Miller D, Jones DB, Bruckner D, Langston R, Jeng BH. *Acanthamoeba* keratitis: The persistence of cases following a multistate outbreak. *Ophthalmic Epidemiology* 2012; 19: 221 - 225.
9. Young G, Veys J, Pritchard N, Coleman S. A multi-centre study of lapsed contact lens wearers. *Ophthalmic and Physiological Optics* 2002; 22: 516 - 527
10. Edwards K, Keay L, Naduvilath T, Snibson G, Taylor H, Stapleton F. Characteristics of and risk factors for contact lens-related microbial keratitis in a tertiary referral hospital. *Eye (Lond)* 2009; 23: 153 - 160.
11. Willcox M, Sankaridurg P, Zhu H, Hume EB, Cole N, Conibear T, Glasson M, Stapleton F. Inflammation and infection and the effects of the closed eye. In: Sweeney D, ed. *Silicone Hydrogels: Continuous wear contact lenses*, 2nd ed. Oxford, UK: Butterworth-Heinemann; 2004; 90 - 125.
12. Keay I, Edwards K., Naduvilath T, Forde K, Stapleton F. Factors affecting the morbidity of contact lens-related microbial keratitis: A population study. *Investigative Ophthalmology and Vision Science* 2006; 47: 4302 - 4308.
13. Dutta D, Cole N, Willcox M. Factors influencing bacterial adhesion to contact lenses. *Molecular Vision* 2012; 18: 14 - 21.
14. Vijay AK, Zhu H, Ozkan J, Wu D, Masoudi S, Bandera R, Borazjani RN, Willcox DP. Bacterial Adhesion to Unworn and Worn Silicone Hydrogel Lenses. *Optometry and Vision Science* 2012; 89 (8): 1095 - 1106.
15. Mayo MS, Schlitzer RL, Ward MA et al. Association of *Pseudomonas* and *Serratia*, corneal ulcers with use of contaminated solutions. *Journal of Clinical Microbiology* 1987; 25: 1398 - 1400.
16. Wilson LA, Schlitzer RL, Ahearn DG. *Pseudomonas* corneal ulcers associated with soft contact lens wear. *American Journal of Ophthalmology* 1981; 92: 546 - 554.
17. O'Brien TP. Management of bacterial keratitis: beyond exorcism towards consideration of organisms and host factors. *Eye (Lond)* 2003; 17: 957 - 974.
18. Hoyle BD, Costerton JW. Bacterial resistance to antibiotics: the role of biofilms. *Progress in Drug Research* 1991; 37: 91- 105.
19. Sankaridurg PR, Willcox MD, Sharma S et al. *Haemophilus influenzae* adherent to contact lenses associated with production of acute ocular inflammation. *Journal of Clinical Microbiology* 1996; 34: 2426 - 2431.

associated with microbial keratitis, contact lens acute red eye,¹⁹ contact lens peripheral ulcers, and certain inflammatory keratitis events.²⁰ The pathogenesis of microbial keratitis includes the adhered bacteria to the biomaterial binding to the corneal epithelium, followed by bacterial invasion into the corneal stroma, releasing inflammatory agents and initiating infection and inflammation.²¹ The purpose of this study was to investigate adhesion of bacteria to worn and unworn silicone hydrogel and conventional hydrogel lenses.

Methods

Clinical procedure

The prospective quasi-experimental design was adopted for this study. A total of thirty (n=30) contact lens wearers of mean age 25.1 ± 1.62 years (22 to 27 years) comprising 13 males and 17 females were recruited for this study. All subjects attended four scheduled visits to have their ocular health and contact lens fitting assessed, as well as contact lens dispensed. Visual acuity testing, preliminary external and internal examination, objective and subjective refraction and keratometry were performed on all the subjects. The refractive status of each subject was noted, and lenses were ordered for each of them in different materials. The tear film stability (as non-invasive tear break-up time) was assessed and those that had less than 10 sec were excluded from the study. Tear flow rate, corneal dimensions, palpebral aperture, and pupil size were also assessed. Written informed consent was obtained from each subject after the procedure and possible outcome were explained. The subjects were given a 2-week supply of each lens type and

lens wear was bilateral and the order of wear of each lens material for each subject was randomized. Contact lenses were worn on a daily disposable basis. Lenses were worn for a minimum of 6 h per day; the lenses were collected on the same day of wear, stored dry in airtight lens cases and then refrigerated (4°C) to avoid contamination. Subjects were advised to wash their hands thoroughly with soap and dry them properly with a clean, dry cloth before handling lenses. A minimum of four pairs of lenses (two per week) were collected from each subject per lens material before performing adhesion assays. Subjects were then given a 2-week supply of the next lens type using randomized allocation after sufficient number of previous lenses was collected. Subjects were strictly instructed not to use any contact lens storage or disinfecting solutions. The research protocol was approved by the departmental research and ethics committee of the University of Benin. The study adhered to the tenets of the Declaration of Helsinki and that of the ethics approval board.

Microorganisms and Culture Preparation

Staphylococcus aureus ATCC 25923 (American type culture collection) and *Pseudomonas aeruginosa* ATCC 27853 obtained from stock culture were used in the adhesion experiment. After growth on chocolate agar plates, strains were grown over night at 37°C in 10 ml of minimal medium for viable adhesion. The bacterial cells were collected and washed three times by centrifugation and suspended in sterile phosphate buffered saline (PBS). The concentration of the bacterial suspension was adjusted to 0.1 at 660 nm using a spectrophotometer (1100RS, Unico Instruments, Cambridge, UK, $\sim 1.0 \times 10^8$ CFU/ml). The suspension was diluted

20. Sankaridurg P, Holden B, Jalbert I. Adverse events and Infections: which ones and how many? In: Sweeney, D, ed. *Silicone Hydrogels: Continuous-wear contact lenses*, 2nd ed. Oxford, UK: Butterworth-Heinemann 2004: 217 - 274.

21. Willcox MDP. New strategies to prevent *Pseudomonas* keratitis. *Eye Contact Lens* 2007; 33: 401 - 403.

in Phosphate buffered saline to obtain the final concentration of 1.0×10^7 CFU/ml which was used for the bacterial adhesion assay.

Preparation of phosphate buffered saline.

Phosphate buffered saline (PBS) with pH 7.4 is a water-based salt solution containing sodium phosphate, sodium chloride and, in some formulations, potassium chloride and potassium phosphate (PBS; NaCl, 8.0 g/l; KCl, 0.2g/l; Na₂HPO₄, 1.42 g/l; KH₂PO₄, 0.24 g/l; pH 7.4). The osmolality and ion concentrations of the solutions match those of the human body. PBS comes in tablets and it was prepared according to manufacturer's instruction of one tablet in 200 ml of sterilized water and after which it was allowed to dissolve and cooled only then was it used for the adhesion assay. For this research PBS was used in washing the worn and unworn contact lenses that were used for adhesion assay and also in serial dilution and vortexing of the washed lenses after incubation and washing. It was however mostly used during the research work because of its pH level which closely approximates the pre-corneal tear film and as such does not affect the assay in anyway.

Bacterial Adhesion Assay

The contact lenses (both worn and unworn) were washed with PBS, and thereafter placed on bacterial - infected plates which in the case of this research were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The washing of the lenses was done using a 2 ml syringe to take up the PBS then with pressure applied as the liquid leaves the syringe, the optical surface i.e., the part that touches the eye of the wearer is washed by means of the pressure exerted by releasing the PBS from the syringe and this was done three times for each contact lens washed. The lenses were incubated for 18 h at 37°C after which the infected lenses were washed with PBS and vortexed with a vortexing machine at the speed

of 10 m/s. The vortexed solution from the contact lenses were serially diluted and the solutions were cultured in nutrient agar in triplicate and incubated for 18 h at 37°C, and colonies grown were thereafter counted with a colony counter. Six unworn lenses and 24 worn lenses of each lens material – Silicone hydrogel (Lotrafilcon A and Lotrafilcon B) lenses and Conventional lenses were used in the adhesion experiments.

The procedures for the bacterial adhesion were carried out at the Microbiology section of the Lahor Research laboratory and Medical Centre, Edo State, Nigeria and all the rules and regulations of the laboratory were strictly adhered to during the assay. Also, the assay was carried out in a sterile cabinet which does not allow air-borne organisms to contaminate the assay as it works at a temperature of 25°C at which airborne organisms cannot survive or contaminate the assay. The following steps were used in the bacterial adhesion assay:

The front surface of the unworn and worn lenses were washed three times each in 2 ml of PBS using a syringe. Thereafter, the washed lenses were aseptically transferred to individual wells of a 24-well cell culture plate containing 1 ml of the bacterial suspension prepared as mentioned above and incubated at 37°C for 18 h, absolute care was taken to avoid contamination of the lenses while washing and placing them on the plate. After the lenses were incubated for 18 h, they were removed aseptically and washed three times in PBS, and placed in sterile universal tubes containing 2 ml of PBS and were vortexed for 2 min at high speed of 10 m/s to detach adhered bacterial cells. The dislodged bacterial suspension after vortexing was serially diluted 10-fold in PBS. Aliquot of each dilution was then inoculated on nutrient agar plates in triplicates and incubated again at 37°C for 18 h. After incubation, the number of viable bacteria per lens was calculated in colony forming units per millimeter (cfu/ml) using a colony counter which

gave a more enlarged view of the colonies formed. The colonies were counted bearing in mind that contaminants are an over growth of the colonies and as such were not counted.

The microorganism and culture preparation as well

as adhesion assay were the same for silicone hydrogel (SiHy) lenses (Lotrafalcon A and Lotrafalcon B) and conventional lenses (polymacon , etafilcon A, nesofilcon A, nelfilcon A, and omafilcon A). Table 1.0 show the contact lens materials used in this study.

Table 1.0 Contact lens materials used in this study

Lens name	Manufacturer	Lens Material (FDA Group)	Water content %	Oxygen Permeability (Dk)
Air Optix Aqua	Alcon laboratory	Lotrafalcon A	24	140
Focus Night & Day	Alcon laboratory	Lotrafalcon B	33	110
1-day Acuvue Moist	Johnson & Johnson	Etafilcon A (IV)	55	28
Biotrue 1-day	Bausch & Lomb	Nesofilcon A (II)	78	42
Focus Dailies Aqua	Alcon	Nelfilcon A (II)	69	26
Proclear 1-Day	Cooper Vision	Omafilcon A (II)	60	28
Impression	Bescon Ltd	Polymacon (I)	38	09

Food Drug Administration classification of lens materials is in bracket after lens material; Groups I & II are nonionic low and high water content (WC); Group IV is ionic high WC soft lens materials; lotrafalcon A and lotrafalcon B are silicon hydrogel (SiHy) lenses.

Data analysis

The statistical package for the social sciences (SPSS ver., 22.0: SPSS Corp., Chicago, IL, USA) for PC was employed for data analysis. The colony forming units (cfu/ml) were transformed to \log_{10} for parametric test statistics to be applied for data analysis. The descriptive statistic was used to obtain measures of central tendencies, standard deviation, standard error and confidence interval. The one-way analysis of variance (ANOVA) with Bonferroni post hoc test (Bonferroni corrected pairwise t-test) was engaged in the comparison of variables across different contact lens materials and

multiple comparisons; Unpaired t-test was used to compare two groups of independent variables. Statistical significance was declared when p-value was ≤ 0.05 .

Results

Bacterial adhesion to worn and unworn extended wear lenses

Two types of silicone hydrogel soft contact lenses - Lotrafalcon A and Lotrafalcon B and five types of conventional (hydroxyethylmethacrylate-based) lenses were used in this study (Table 2.0)

Table 2.0 Bacterial adhesion to contact lens materials

Contact lens material	<i>P. aeruginosa</i> on worn lenses	<i>P. aeruginosa</i> on unworn lenses	<i>S. aureus</i> on worn lenses	<i>S. aureus</i> on unworn lenses
Lotrafilcon A	8.32 ± 0.56	8.43 ± 0.09	7.00 ± 1.26	8.15 ± 0.22
Lotrafilcon B	8.28 ± 0.37	7.82 ± 0.27	8.18 ± 0.26	8.14 ± 0.07
Polymacon	8.13 ± 0.36	7.77 ± 0.08	8.02 ± 0.35	7.72 ± 0.12
Nelfilcon A	8.53 ± 0.35	7.95 ± 0.05	7.47 ± 0.38	7.80 ± 0.07
Nesofilcon A	8.62 ± 0.22	8.14 ± 0.09	7.62 ± 0.44	7.99 ± 0.03
Etafilcon A	8.67 ± 0.36	8.07 ± 0.10	7.35 ± 0.36	8.33 ± 0.09
Omafilcon A	8.61 ± 0.27	7.75 ± 0.05	7.22 ± 0.11	7.72 ± 0.05

The cfu/ml values were transformed to log₁₀, i.e, log₁₀ cfu/lens.

For worn lenses, the difference in mean adhesion of *P.aeruginosa* between Lotrafilcon A (8.32 ± 0.56, CI ± 0.16) and Lotrafilcon B (8.28 ± 0.37, CI ± 0.11) was not statistically significant (p = 0.85). However, the difference in mean adhesion of *Staphylococcus aureus* between lotrafilcon A (7.00 ± 1.26, CI ± 0.39) and lotrafilcon B (8.18 ± 0.26, CI ± 0.08) was statistically significant (p = 0.009). The difference in mean adhesion of *Pseudomonas aeruginosa* between unworn Lotrafilcon A (8.43 ± 0.09; CI ± 0.04) and Lotrafilcon B (7.82 ± 0.27; CI ± 0.11) was significant (P = 0.009). Meanwhile, the difference in mean adhesion of *Staphylococcus aureus* between unworn Lotrafilcon A (8.15 ± 0.26; CI ± 0.11) and Lotrafilcon B (8.14 ± 0.07; CI ± 0.03) was not significant (p = 0.90).

Worn and Unworn Contact lenses

For Lotrafilcon A, the difference in mean adhesion of *Pseudomonas aeruginosa* between worn and unworn lenses was not significant (p = 0.66) (worn: 8.32 ± 0.56; CI ± 0.16 unworn: 8.43 ± 0.09; CI ± 0.04).

For Lotrafilcon B, however, the difference in mean adhesion of *Pseudomonas aeruginosa* between worn lenses (8.28 ± 0.38; CI ± 0.11) and unworn

lenses (7.82 ± 0.27; CI ± 0.11) was also significant (p = 0.018). The difference in mean adhesion of *Staphylococcus aureus* to worn (7.00 ± 1.34; CI ± 0.39) and unworn (8.15 ± 0.26; CI ± 0.11) Lotrafilcon A was significant (p = 0.018). However, the difference in mean adhesion of *Staphylococcus aureus* to worn (8.18 ± 0.26; CI ± 0.08) and unworn (8.14 ± 0.07; CI ± 0.03) lotrafilcon A lenses was not significant (p = 0.74) (Table 3).

Bacterial adhesion to worn and unworn daily wear contact lenses

Pseudomonas aeruginosa Versus *Staphylococcus aureus*

The difference in mean adhesion of 0.37 to Polymacon by *Pseudomonas aeruginosa* between worn (8.14 ± 0.36) and unworn (7.77 ± 0.08) lenses was significant (p = 0.03). However, the difference in mean adhesion (0.29) of *Staphylococcus aureus* to polymacon between worn (8.02 ± 0.35) and unworn (7.73 ± 0.12) was not significant (p = 0.07). The difference in mean adhesion of 0.12 to worn polymacon between *Pseudomonas aeruginosa* (8.14 ± 0.36) and *Staphylococcus aureus* (8.02 ± 0.35) was not significant (p = 0.48). The difference in the mean adhesion of 1.32 between *Pseudomonas aeruginosa* (8.67 ± 0.37) and *Staphylococcus*

aureus (7.35 ± 0.36) to HEMA-based worn etafilcon A was significant ($p < 0.001$). The difference of 1.00 in the mean adhesion of *Pseudomonas aeruginosa* (8.62 ± 0.22) and *Staphylococcus aureus* (7.62 ± 0.44) to worn nesofilcon A was significant ($p < 0.001$). The difference of 1.06 in the mean adhesion of *Pseudomonas aeruginosa* (8.53 ± 0.35) and *Staphylococcus aureus* (7.47 ± 0.38)

to worn Nelfilcon A was statistically significant ($p < 0.001$). The difference of 1.39 in the mean adhesion of *Pseudomonas aeruginosa* (8.61 ± 0.27) and *Staphylococcus aureus* (7.22 ± 0.12) to worn omafilcon A was significant ($p < 0.001$). Contact lens wear showed varied effects on bacterial adhesion to SiHy and conventional lenses (Table 3.0).

Table 3.0 The differences in mean adhesion of Pseudomonas aeruginosa and Staphylococcus aureus to worn and unworn contact lenses of different materials

Lens material	Worn CLs	Unworn CLs	Difference	p-value	Significance
<i>Pseudomonas aeruginosa</i>					
Lotrafilcon A	8.32 ± 0.56	8.43 ± 0.09	0.11	0.66	NS
Lotrafilcon B	8.28 ± 0.38	7.82 ± 0.27	0.56	0.018	S
Polymacon	8.14 ± 0.36	7.77 ± 0.08	0.37	0.03	S
Etafilcon A	8.67 ± 0.36	8.07 ± 0.10	0.60	0.001	S
Nesofilcon A	8.62 ± 0.22	8.14 ± 0.09	0.48	<0.001	S
Nelfilcon A	8.53 ± 0.35	7.95 ± 0.05	0.59	<0.001	S
Omafilcon A	8.61 ± 0.27	7.75 ± 0.05	0.86	<0.001	S
<i>Staphylococcus aureus</i>					
Lotrafilcon A	7.00 ± 1.34	8.15 ± 0.26	1.15	0.018	S
Lotrafilcon B	8.18 ± 0.26	8.14 ± 0.07	0.04	0.74	NS
Polymacon	8.02 ± 0.35	7.73 ± 0.12	0.29	0.07	NS
Etafilcon A	7.35 ± 0.36	8.33 ± 0.09	0.98	<0.001	S
Nesofilcon A	7.62 ± 0.44	7.99 ± 0.03	0.37	0.06	S
Nelfilcon A	7.47 ± 0.38	7.80 ± 0.07	0.33	0.05	S
Omafilcon A	7.22 ± 0.12	7.72 ± 0.05	0.50	<0.001	S

S = Significant; NS = not significant

Lens Material versus Organism

The difference in the mean adhesion of *Pseudomonas aeruginosa* to worn lenses of different materials was statistically significant (ANOVA: $p = 0.001$). Post hoc test with Bonferroni correction showed that Etafilcon A when compared with Nesofilcon A,

Nelfilcon A, and Omafilcon A was not significantly different. However, mean adhesion of *P. aeruginosa* to worn Polymacon lenses was significantly lower than that obtained from etafilcon A, nesofilcon A, nelfilcon A and omafilcon A (0.53, 0.49, 0.39, and 0.48) respectively.

The difference in the mean adhesion of *Staphylococcus aureus* to worn lenses of different materials was statistically significant (ANOVA: $p < 0.001$). Bonferroni correction showed that the adhesion of *S. aureus* to polymacon was significantly higher than etafilcon A (0.67), nesofilcon A (0.40), nelfilcon A (0.56), and omafilconA (0.80) ($p < 0.001$).

Discussion

Bacterial adhesion to contact lenses of different materials is fundamental to the initiation of the cascade of events characterizing the pathogenesis of contact lens-related microbial corneal infection termed microbial keratitis.

The major risk factors for contact-lens-associated microbial keratitis are overnight use of daily wear lenses,²² using lenses on extended wear schedule for longer duration,²³ inadequate hygiene,²² and poor contact lens storage case cleaning.²⁴ The most prevalent gram-negative bacilli majorly implicated in contact lens - associated microbial keratitis is *Pseudomonas aeruginosa*.^{1,22-26} *Pseudomonas aeruginosa* adhered in greater numbers to unworn SiHy (lotrafilcon A) than the hydroxyethyl methacrylate - based materials and this was consistent with the report of Willcox and colleagues.²⁷ SiHy lenses had greater numbers of adhered *Staphylococcus aureus* than the HEMA-based lenses except etafilcon A material that had the greatest numbers. Lens wear significantly reduced the numbers of *Staphylococcus aureus* adhesion to silicone hydrogel and HEMA-based lenses.

The reduction in the numbers of *Staphylococcus aureus* that adhered to worn contact lenses was due to adsorbed lysozyme which is present in high concentration in tear film.¹⁴ Lysozyme is a potent antimicrobial enzyme against Gram-positive bacteria. It exerts its bacteriocidal effect by catalytic hydrolysis of the peptidoglycan component of the cell wall. This it does by breaking the β -(1, 4) glycosidic bond of the oxygen bridge between the repeating glycans units of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). With the splitting open of the peptidoglycan cover of the cell wall, the bacterium is no longer able to contain its high internal osmotic pressure with its plasma membrane alone and it bursts open.²⁸ In all the silicone hydrogel and conventional lenses used in this study, *P. aeruginosa* demonstrated higher extent of adhesion than *Staphylococcus aureus* for the worn lenses and this was in agreement with previous reports.^{1,25,29} Vijay et al.¹⁴ found that strains of *Pseudomonas aeruginosa* adhered in higher numbers than strains of *Staphylococcus aureus* to silicone hydrogel lenses, regardless of lens polymer type or surface properties of the lens. Borazjani et al.²⁹ found no marked difference in the adhesion of *P. aeruginosa* to worn and unworn silicone hydrogel lenses, suggesting that these lens surface properties were not affected by the presence of tear film molecules, especially the antibacterial agent, lactoferrin active against gram-negative bacteria. Henriques et al.³⁰ found a significant higher extent of adhesion of *P. aeruginosa* to the silicone hydrogel lens, with the exception of galyfilcon A.

1. Green M, Apel A, Stepleton, F. Risk factors and causative organisms in microbial keratitis. *Cornea* 2008; 27: 22 - 27.
14. Vijay AK, Zhu H, Ozkan J, Wu D, Masoudi S, Bandera R, Borazjani RN, Willcox DP. Bacterial Adhesion to Unworn and Worn Silicone Hydrogel Lenses. *Optometry and Vision Science* 2012; 89 (8): 1095 - 1106.
22. 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 - 618.
23. Cheng KAJ, Kijlstra A. Incidence of contact lens-associated microbial keratitis and its related morbidity. *Lancet* 1999; 354: 181 - 185.
24. Radford CF, Minassian DC, Dart JK. Disposable contact lens use as a risk factors for microbial keratitis. *British Journal of Ophthalmology* 1998; 82: 1272 - 1275.
25. Sharma S, Gopalakrishnan S, Aasuri MK, Garg P, Rao GN. Trends in contact lens-associated microbial keratitis in Southern India. *Ophthalmology* 2003; 110: 138 - 143.
26. Mah-Sadorra JH, Yavuz SG, Najjar DM, Laibson PR, Rapuano CJ, Cohen EJ. Trends in contact lens-related corneal ulcers. *Cornea* 2005; 24: 51 - 58.
27. Willcox MDP. Microbial adhesion to silicone hydrogel lenses: A review. *Eye & Contact Lens: Science & Clinical Practice* 2013; 39(1): 61 - 66.
28. Whikehart D. *Biochemistry of the Eye*, 2nd ed. Philedelphia, PA: Butterworth-Heinemann/Elsevier. 2003: 319pp.
29. 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 Anterior Eye* 2004; 27: 3 - 8.
30. Henriques M, Sousa C, Lira M, Elisabete M, Oliveira R, Oliveira J, Azeredo J. Adhesion of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* to silicone-hydrogel contact lenses. *Optometry and Vision Science* 2005; 82(6): 446 - 450.

Willcox et al.³¹ also found an increased capability of *P. aeruginosa* to adhere to silicone- hydrogel balafilcon A when compared with the adhesion to conventional hydrogels. These contradictory results may be attributed to different bacterial strains and growth conditions used.³⁰ Subbaraman et al.³² attributed the increase in adhesion of *P. aeruginosa* or *S. aureus* to lactoferrin-coated silicone hydrogel to lactoferrin showing antimicrobial effect against the attached *P. aeruginosa* strains. Lactoferrin interacts with lipopolysaccharide in cell membranes of Gram-negative bacteria, increasing their membrane permeability and leading to eventual death.³³ Conventional hydrogel lens materials, particularly ionic group IV lenses, accumulate high levels of the antibacterial proteins, lysozyme and lactoferrin. If these proteins remain active, then they may have the ability to reduce the viability of adherent gram-positive and gram-negative bacteria, resulting in reduced rates of infiltrative events and possibly microbial keratitis.³⁴ The positive charge and small size of lysozyme result in it having a great affinity for negatively charged, group IV hydrogel lenses, in particular those with relatively high amounts of acidic groups, such as etafilcon A. Ionic hydrogel materials (pH 6.0 - 8.0) accumulate significantly more lysozyme than Silicon hydrogel materials. Most of the activity of lysozyme deposited on etafilcon A is retained and is primarily located

within the bulk of the lens rather than the surface.³⁴ It has been shown that certain silicone hydrogel materials undergo surface modification to improve their wettability and this surface modification influences the amount of lysozyme that deposit on them.³⁵⁻³⁷ Deposition of lysozyme decreases bacterial adhesion to lenses and contact lens wettability is not affected. Lactoferrin is synergistic with lysozyme and has the potential to reduce the viability of gram-negative and gram-positive bacteria which are implicated in the pathogenesis of microbial keratitis and inflammation. The ability of *P. aeruginosa* to adhere in high numbers to many contact lens types may be one of the reasons that the bacterium is the predominant causative microbe for contact-lens-related microbial keratitis.^{1,22,25,38,39} Williams et al.⁴⁰ reported that lens wear generally increased adhesion of total cells but decreased viable adhesion, for a strain (Paer1) of *P. aeruginosa* to etafilcon A lenses. Vijay et al.¹⁴ asserted that the effect of lens wear on adhesion may be due to differences between strains. In their study, lens wear decreased viable cells adhesion to galyfilcon A, lotrafilcon B, and lotrafilcon A lenses ($p < 0.05$), but enhanced viable cells adhesion to balafilcon A lenses ($p < 0.05$). The addition of tear components, such as lysozyme, lactoferrin, albumin to hydroxymethyl methacrylate-based contact lens, increased adhesion.⁴¹ Lactoferrin deposited on

1. Green M, Apel A, Stepleton, F. Risk factors and causative organisms in microbial keratitis. *Cornea* 2008; 27: 22 - 27.
14. Vijay AK, Zhu H, Ozkan J, Wu D, Masoudi S, Bandera R, Borazjani RN, Willcox DP. Bacterial Adhesion to Unworn and Worn Silicone Hydrogel Lenses. *Optometry and Vision Science* 2012; 89 (8): 1095 - 1106.
22. 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 - 618.
25. Sharma S, Gopalakrishnan S, Aasuri MK, Garg P, Rao GN. Trends in contact lens-associated microbial keratitis in Southern India. *Ophthalmology* 2003; 110: 138 - 143.
30. Henriques M, Sousa C, Lira M, Elisabete M, Oliveira R, Azeredo J. Adhesion of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* to silicone-hydrogel contact lenses. *Optometry and Vision Science* 2005; 82(6): 446 - 450.
31. Willcox MDP, Harmis N, Cowell BA, Williams T, Holden BA. Bacterial interactions with contact lenses; effects of lens material, lens wear and microbial physiology. *Biomaterials* 2001; 22: 3235 - 3237.
32. Subbaraman LN, Borazjani R, Zhu H, Zhao Z, Jones L, Willcox MD. Influence of protein deposition on bacterial adhesion to contact lenses. *Optometry and Vision Science* 2011; 88: 959 - 966.
33. Leitch EC, Willcox MD. Synergic antistaphylococcal properties of lactoferrin and lysozyme. *Journal of Medical Microbiology* 1998; 47: 837 - 842.
34. Babaei Omali N, Subbaraman LN, Cole-Brennan C, Fadli Z, Jones LW. Biological and clinical implications of lysozyme deposition on soft contact lenses. *Optometry and Vision Science* 2015; 92(7): 750 - 757.
35. Jones L, Senchyna M, Glasier MA, Schickler J, Forbes I, Louie D, May C. Lysozyme and lipid deposition on silicone hydrogel contact lens materials. *Eye & Contact Lens* 2003; 29: S75 - S79.
36. Subbaraman LN, Glasier MA, Senchyna M, Sheardown H, Jones L. Kinetics of in vivo lysozyme deposition on silicone hydrogel, PMMA, and FDA groups I, II and IV contact lens materials. *Current Eye Research* 2006; 31: 787 - 796.
37. Suwala M, Glasier MA, Subbaraman LN, Jones L. Quantity and conformation of lysozyme deposited on conventional and silicone hydrogel contact lens materials using an in vivo model. *Eye Contact Lens* 2007; 33: 138 - 143.
38. Bourcier T, Thomas F, Borderie V, Chaumeil C, Loroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *British Journal of Ophthalmology* 2003; 87: 834 - 838.
39. Pachigolla G, Blomquist P, Cavanagh HD. (2007). Microbial keratitis pathogens and antibiotic susceptibilities: a 5-year review of cases at an urban county hospital in north Texas. *Eye Contact Lens* 2007; 33: 45 - 49.
40. Williams TJ, Schneider RP, Willcox MD. The effect of protein-coated contact lenses on the adhesion and viability of gram-negative bacteria. *Current Eye Research* 2003; 27: 227 - 235.
41. Butrus S.I, Klotz SA, Misra RP. The adherence of *Pseudomonas aeruginosa* to soft contact lenses. *Ophthalmology* 1987; 94: 1310 - 1314.

either HEMA-based or silicone hydrogel contact lenses can decrease the viability of adhered *P. aeruginosa*.^{32,40} The adhesion to contact lenses in vitro varied with the type of lens, polymer, bacterial genus with *P. aeruginosa* usually adhering to lenses in greater numbers than other genera/species, or species, or strain or indeed the environmental conditions individual strains were grown under. *P. aeruginosa*, once adhered to a contact lens, could utilize the adsorbed tear film components (proteins, lipids, mucin) for growth.³¹ The lenses used for this study belong to United States Federal Food and Drug Administration (FDA) classification/group-groups I (nonionic polymer, <50% low water content, e.g., Polymacon), II (nonionic polymer, >50% High water content, e.g., nelfilcon A, nesofilcon A, omafilcon A), IV, (ionic polymer, >50% high water content, e.g., etafilcon A), similar to the FDA groups of hydrogel lenses, Subbaraman et al.³⁶ used in their study. In the present study, however, there was no correlation done between low water content lenses and high-water content lenses. However, the low water content lenses (Polymacon) showed relatively low adhesion to the two bacterial types (both worn and unworn) as compared to the high-water content lenses (nesofilcon A, nelfilcon A and omafilcon A) which showed higher bacterial adhesion.

The effect of lens material on bacterial adhesion has been studied previously although all the materials investigated by this study may not have been fully discussed by others. The difference in the mean adhesion of *Pseudomonas aeruginosa* to worn lenses of different materials was statistically significant. Etafilcon A when compared with

nesofilcon A, nelfilcon A and omafilcon A was not significantly different. But mean adhesion of *Pseudomonas aeruginosa* to Polymacon was significantly lower than etafilcon A, nesofilcon A, nelfilcon A and omafilcon A. For *Staphylococcus aureus*, the difference in mean adhesion of *Staphylococcus aureus* to worn lenses of different materials was statistically significant. Adhesion of *Staphylococcus aureus* to Polymacon was significantly higher than mean adhesion to etafilcon A, nesofilcon A, nelfilconA, and omafilcon A. Etafilcon A demonstrated the highest change in adhesion of *Staphylococcus aureus*, with lower value after lens wear due to adsorbed and absorbed lysozyme from the tear film, which could be a direct function of the lens charge density (ionicity) and porosity (water content), since it belongs to group IV of FDA classification of hydrogel lenses. This finding was consistent with the claims of Garrett and colleague.⁴²

When comparing adhesion rates in relation to oxygen permeability, no correlation was done. However, nesofilcon A had the highest oxygen permeability (Dk = 42) and yet no significant increase or decrease in adhesion seen when compared to the other lenses. Polymacon, with lowest oxygen permeability (Dk = 09) had the highest adhesion of *Staphylococcus aureus* than worn etafilcon A, nesofilcon A, nelfilcon A and omafilcon A. While for *Pseudomonas aeruginosa*, the reverse was the case. Mean adhesion of *Pseudomonas aeruginosa* to Polymacon was significantly lower than that obtained for worn etafilcon A, nesofilcon A, nelfilcon A and omafilcon A. Hydroxyethyl

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31. Willcox MDP, Harmis N, Cowell BA, Williams T, Holden BA. Bacterial interactions with contact lenses; effects of lens material, lens wear and microbial physiology. *Biomaterials* 2001; 22: 3235 - 3237.
32. Subbaraman LN, Borazjani R, Zhu H, Zhao Z, Jones L, Willcox MD. Influence of protein deposition on bacterial adhesion to contact lenses. *Optometry and Vision Science* 2011; 88: 959 - 966.
36. Subbaraman LN, Glasier MA, Senchyna M, Sheardown H, Jones L. Kinetics of in vivo lysozyme deposition on silicone hydrogel, PMMA, and FDA groups I, II and IV contact lens materials. *Current Eye Research* 2006; 31: 787 - 796.
40. Williams TJ, Schneider RP, Willcox MD. The effect of protein-coated contact lenses on the adhesion and viability of gram-negative bacteria. *Current Eye Research* 2003; 27: 227 - 235.
41. Butrus S.I, Klotz SA, Misra RP. The adherence of *Pseudomonas aeruginosa* to soft contact lenses. *Ophthalmology* 1987; 94: 1310 - 1314.
42. Garrett Q, Garrett RW, Milthorpe BK. Lysozyme absorption in hydrogel contact lenses. *Investigative Ophthalmology and Vision Science* 1999; 40: 897 - 903.

methacrylate-based lenses have greater affinity for *Pseudomonas aeruginosa*.

The sample size for this study was small, which is the major limitation encountered in this study. Despite this shortcoming, the study was able to show that lens wear had effect on the adhesion of the bacteria cells to lenses of different materials. While lens wear decreased the number of *S. aureus*

to lotrafilcon A, etafilcon A, nesofilcon A, nelfilcon A, and omafilcon A, higher number of *P. aeruginosa* adhered to Lotrafilcon A, polymacon, etafilcon A, nesofilcon A, nelfilcon A and omafilcon A. This provides further understanding to the implication of *P. aeruginosa* in majority of cases of bacterial keratitis associated with contact lens wear.

Conclusion

From this study, lens wear has been shown to have different effects on bacterial adhesion, which may be due to type of lens materials and bacterial species/genera studied. The numbers of *Pseudomonas aeruginosa* that adhered to worn polymacon was higher compared to nelfilcon A, nesofilcon A, etafilcon A and omafilcon A, while *Staphylococcus aureus* adhered more to worn polymacon than the other conventional lens materials. *Pseudomonas aeruginosa* adhered equally to worn silicone hydrogel lenses, but significantly lower numbers of *Staphylococcus aureus* adhered to Lotrafilcon A than Lotrafilcon B. The lower the adherence of the bacterial load to a lens material, the lower the susceptibility of the lens material to harbour the amount of organism that would initiate the series of events that could result in bacterial infection through the invasion of the corneal tissue when there is epithelial defect of the cornea.

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