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Comparative Evaluation of Bacterial Adhesion and Biofilm Formation on Contact Lenses

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ABSTRACT

This study evaluates bacterial adhesion, biofilm formation, and Staphylococcus aureus (S. aureus) (ATCC 25923) colonization on medical and cosmetic contact lenses made from Hilafilcon B and Omafilcon B under simulated ocular conditions. Non-incubated lenses served as controls, and experiments were repeated three times with a variability margin of \pm 5%, ensuring accuracy and reliability. Results revealed that biofilm growth and bacterial adhesion were influenced by lens material, type, and incubation duration. Cosmetic lenses made from Hilafilcon B exhibited the highest biofilm growth (1.95%) after one day, highlighting their susceptibility to microbial colonization. Conversely, medical lenses made from Hilafilcon B demonstrated the lowest biofilm growth (0.63%) after seven days, indicating potential inhibitory effects on bacterial adaptation. In terms of bacterial adhesion, Omafilcon B showed lower colonization at intermediate periods, with the lowest S. aureus adhesion observed on medical Omafilcon B lenses (64.38%) after 28 days. Antibacterial contact lenses exhibited strong inhibitory effects, with zones of inhibition ranging from 15-20 mm, confirming their effectiveness in reducing microbial colonization. The interaction between lens surface hydrophilicity and bacterial adhesion was a major factor, with ionic materials demonstrating higher biofilm formation due to increased electrostatic attraction. These findings emphasize the critical role of lens material properties, such as water content and ionic charge, in modulating bacterial interactions. The study underscores the importance of selecting appropriate lens materials and implementing advanced hygiene protocols to minimize microbial risks, offering valuable insights for designing safer and more effective contact lenses.

Keywords: Antibacterial contact lenses, *Staphylococcus aureus*, Hilafilcon B, Omafilcon B, Ocular microbiology, Tear film interaction

1. Introduction

The widespread use of contact lenses, both cosmetic and medical, has raised concerns regarding their susceptibility to microbial colonization, particularly by *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Bacterial adhesion and subsequent biofilm formation can lead to severe ocular infections. However, these lenses also come with an increased risk of ocular infections, particularly when hygiene practices are inadequate or when lenses are purchased without a prescription. A review of multiple studies, summarized by Lim et al [1], explores various documented cases and epidemiological studies on cosmetic contact lens infections, highlighting key pathogens, prevalence, and risk factors. Cosmetic lenses, designed for aesthetic purposes, may reduce oxygen permeability due to pigments, increasing the risk of microbial adhesion and contamination [2]. Recent studies have highlighted the importance of advanced strategies, such as selfassembled nanoparticles in ocular delivery, which offer promising approaches to mitigate microbial colonization and enhance ocular health in cosmetic contact lens users [3]. Pathogens like Pseudomonas aeruginosa, Acanthamoeba [4], and *S. aureus* are

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commonly associated, potentially leading to severe infections like keratitis [5].

Conventional lenses are primarily prescribed for vision correction and regulated as medical devices, requiring prescriptions and professional fitting [6]. Made from oxygen-permeable materials like silicone hydrogels, they lower infection risks with proper cleaning routines [7]. However, users remain vulnerable to pathogens like *Pseudomonas aeruginosa*, *Fusarium, Acanthamoeba*, and *Staphylococcus aureus*, which can cause infections if lenses are mishandled [8], or exposed to contaminants [9].

Cosmetic contact lenses, designed to alter or enhance eve appearance, have gained significant popularity worldwide [10]. Their usage is particularly prevalent in countries with strong fashion and beauty industries. In Asia, nations like South Korea, Japan, and China lead in cosmetic lens adoption, with consumers frequently using them to complement their aesthetic preferences [11]. In the United States, the cosmetic contact lens market is substantial, accounting for approximately 73.7% of the total market share in North America as of 2023 [12]. European countries, including Germany and the United Kingdom, also exhibit notable usage, driven by fashion trends and a growing emphasis on personal appearance [13]. This widespread adoption underscores the importance of understanding and mitigating the associated risks of ocular infections linked to cosmetic contact lens use.

Bacteria are the most common pathogens causing infections in contact lens wearers, with Pseudomonas aeruginosa (P. aeruginosa) and S. aureus being particularly notable. These bacteria thrive on lens surfaces, binding to them, and forming biofilms that resist antibiotics and disinfectants. Biofilms can facilitate prolonged bacterial adhesion, leading to infections such as microbial keratitis [14], characterized by corneal ulcers [15], redness [16], and vision impairment [17]. Mark D.P. Willcox provided [18], in his review, an in-depth analysis of P. aeruginosa as a primary pathogen in contact lens-related infections [19]. In 2020, Raksha L. conducted a study on biofilm formation in bacterial isolates from contact lens wearers [20]. The study analyzed biofilm production in 265 bacterial isolates from conjunctiva, contact lenses, and storage cases using phenotypic and genotypic methods, identifying S. aureus, and Pseudomonas aeruginosa. Over 50% of isolates exhibited biofilm production, highlighting significant infection risks. Concurrently, research by Shen et al. examined P. aeruginosa sensitivity to disinfectants and microbial adhesion on worn cosmetic contact lenses [21]. The study evaluated disinfectant efficacy on P. aeruginosa genotypes across Etafilcon, Nelfilcon,

and Hilafilcon lenses, finding Hilafilcon most prone to contamination and resistant to Renu Fresh disinfectant. Type I [22], and II systems are involved in general protein and toxin secretion [23], while Type III functions as a specialized needle-like apparatus that injects virulence factors directly into host cells, significantly contributing to bacterial pathogenicity and biofilm formation [23].

The objective of this study is to systematically investigate the antibacterial efficacy, bacterial adhesion, and biofilm formation on medical and cosmetic contact lenses. Lens materials and types, specifically Hilafilcon B and Omafilcon B, were selected based on insights from an extensive questionnaire conducted with contact lens users to ensure real-world relevance. The study focuses on evaluating bacterial interactions using hydrogel discs under controlled conditions, with particular emphasis on the Grampositive bacterial strain *Staphylococcus aureus* (ATCC 25923).

2. Materials and methods

2.1. Contact lens selection

This study investigates the antibacterial efficacy, bacterial adhesion, and biofilm formation on contact lenses using a systematic experimental approach. Lens materials and types were chosen following an extensive questionnaire conducted with contact lens users, ensuring real-world relevance. The questionnaire gathered information on contact lens usage patterns, lens types, wear duration, and any lensassociated eye issues experienced by the users (see S1 in Appendix A). The detailed information of Hilafilcon B and Omafilcon B contact lenses are listed in Table 1. Medical and cosmetic contact lenses made from Hilafilcon B and Omafilcon B were evaluated, with hydrogel discs used to assess bacterial interactions under controlled conditions, including incubation at 37°C to simulate ocular temperature, exposure to artificial tear solution to mimic the tear film environment, and standardized bacterial inoculation with S. aureus (ATCC 25923). The discs were incubated in a shaker incubator at 60 rpm to maintain consistent bacterial exposure and prevent sedimentation, with all experiments conducted in sterile environments to ensure experimental integrity. The primary focus was on the Gram-positive bacterial strain S. aureus (ATCC 25923). This strain was chosen due to its clinical relevance as a common ocular pathogen associated with contact lens-related infections. S. aureus is known for its strong biofilm-forming capabilities and its ability to adhere to contact lens materials, making it an ideal model organism for

Brand	Refractive Index	No. of participants	*O ₂ permeability	*Equilibrium water content (%)	Material	**Base polymer
Proclear	1.390	166	25	62	Omafilcon B	HEMA
Soflens Daily	1.403	613	19	59	Hilafilcon B	PVP

Table 1. The selected contact lenses detailed information based on data obtained directly from the manufacturers' product datasheets.

*Food and Drug Administration (FDA) classification of contact lenses based on material properties and water content: Group I (non-ionic, low water content <50%), Group II (non-ionic, high water content >50%), and Group IV (ionic, high water content >50%). These classifications influence the lenses' interaction with the tear film and microbial colonization dynamics. Lenses made from materials like Hilafilcon B (FDA Group IV – ionic, high water content) and Omafilcon B (FDA Group II – non-ionic, high water content).

** Hydroxyethyl Methacrylate (HEMA), Polyvinylpyrrolidone (PVP).

evaluating bacterial adhesion and biofilm formation. The ATCC 25923 strain is a widely used standard in antimicrobial and adhesion studies, ensuring reproducibility and comparability with existing literature [24]. This rationale has been incorporated to clarify the choice of the bacterial strain.

2.2. Methodology

The experimental procedures for assessing biofilm growth trends [25], bacterial adhesion [26], and *S. aureus* colonization on contact lenses were conducted systematically by following standardized protocols, maintaining controlled conditions (37° C, 60 rpm), using sterile techniques, and repeating each test three times with non-incubated lenses as controls to ensure consistency and reproducibility. This is necessary to evaluate microbial interactions with different lens materials, with non-incubated lenses serving as controls.

For biofilm growth trends, hydrogel discs punched from Hilafilcon B and Omafilcon B lenses were hydrated at 37°C for 24 hours in a sterile environment and then exposed to a 50:50 mixture of Alcon Tears Naturale II Med Lubricating Eye Drops (TEARS NATURALE®) and S. aureus (ATCC 25923). Biofilm growth was measured using optical density at 600 nm with a DeNovix DS-C Spectrophotometer, calibrated according to the manufacturer's instructions before each measurement to ensure accuracy. Biofilm formation was further visualized through microscopy over incubation periods of 1, 7, 14, and 28 days. Percentage growth in biofilm biomass over time was quantified using optical density readings and validated through microscopy imaging to confirm structural biofilm development.

To assess bacterial adhesion, the hydrated discs were incubated with *S. aureus* in Luria Broth at 37°C for the same time points, followed by washing with Phosphate-Buffered Saline (PBS) to remove non-adherent bacteria [27]. Adhered bacteria were recovered by vortexing or sonication and quantified through colony-forming unit (CFU) counts [28]. For *S. aureus* colonization, the discs were incubated in a 50:50 bacterial medium and artificial tear solution at 37°C for 5 hours to simulate ocular conditions.

Colonization levels were quantified through CFU counts, and the Kirby Bauer method was applied to evaluate antibacterial activity by measuring zones of inhibition [29]. Each test was repeated three times, and variability was maintained within $\pm 5\%$, calculated based on the standard deviation from triplicate measurements. Controlled environmental conditions, including consistent incubation temperature, agitation speed, and sterile handling, were employed to ensure accuracy and reliability of the results. This approach provided comprehensive insights into bacterial colonization dynamics and lens material performance, ensuring reproducibility and minimizing experimental error.

3. Results and discussions

3.1. Biofilm growth trends

The biofilm growth trends observed across incubation periods, lens types, and materials reveal significant interactions that influence bacterial colonization. Initially, non-incubated lenses show low biofilm growth, with medical lenses made from Omafilcon B exhibiting the least growth (0.78%), indicating an initial resistance to bacterial adhesion [30], as shown in Fig. 1a. However, after one day of incubation, biofilm growth increases notably, particularly on cosmetic lenses with Hilafilcon B (1.95%) as shown in Fig. 1b, highlighting the material's susceptibility to microbial colonization. By the seventh day, biofilm growth on medical lenses drops, especially with Hilafilcon B (0.63%), suggesting potential inhibitory effects or adaptation challenges for bacteria %) as shown in Fig. 1a. Conversely, cosmetic lenses maintain higher growth, especially on Omafilcon B (1.88%), indicating material-specific differences in supporting bacterial biofilm. At 14 days,



Fig. 1. Biofilm growth (%) on contact lenses of different types (a. medical and b. cosmetic) and materials (Hilafilcon B and Omafilcon B) across incubation periods.

biofilm formation declines across most conditions, likely due to nutrient depletion or bacterial competition, with the lowest growth observed on cosmetic lenses with Hilafilcon B (0.57%) %) as shown in Fig. 1b. Interestingly, by 28 days, biofilm growth resurges, particularly on cosmetic lenses with Hilafilcon B (1.74%) and medical lenses with Omafilcon B (1.31%), demonstrating bacterial adaptation and biofilm maturation as shown in Fig. 1a. These findings suggest that lens type, material composition, and wear duration significantly impact biofilm dynamics, with cosmetic lenses being more prone to biofilm formation and Hilafilcon B showing higher susceptibility overall. This underscores the need for material optimization, improved hygiene protocols, and targeted interventions to mitigate microbial risks associated with contact lens use.

The initial decrease in biofilm growth after 14 days can be attributed to nutrient depletion and bacterial competition within the biofilm structure. As bacteria proliferate and consume available nutrients, limited resources lead to reduced biofilm biomass and potential detachment of non-viable cells. Additionally, bacterial competition within the biofilm can cause certain populations to die off or enter a dormant state, further contributing to the decline in biomass.

The subsequent increase in biofilm growth observed at 28 days is likely due to bacterial adaptation mechanisms and biofilm maturation. Over time, biofilms develop complex structures that facilitate nutrient recycling and create protective microenvironments. Mechanisms such as quorum sensing may activate biofilm growth-promoting genes, leading to increased biomass even under nutrient-limited conditions. In Hilafilcon B lenses, the ionic nature and surface properties may further support biofilm resurgence by promoting bacterial attachment and retention. Similarly, for Omafilcon B, its hydrophilic nature might allow better nutrient absorption during extended incubation, contributing to biofilm regrowth.

The ionic nature of Hilafilcon B lenses is classified based on the FDA contact lens grouping system, where Hilafilcon B falls under FDA Group IV, which includes ionic materials with high water content (>50%). This classification is well-established in ophthalmic literature and indicates that Hilafilcon B lenses possess negatively charged ionic groups on their surface. These ionic characteristics promote bacterial adhesion through electrostatic interactions with positively charged regions on bacterial cell walls, particularly in *S. aureus* [31]. This electrostatic attraction, combined with the hydrophilic and highwater-content nature of the lens, makes Hilafilcon B more susceptible to bacterial colonization and biofilm formation.

3.2. Bacterial adhesion

The bacterial adhesion trends observed on medical lenses made from Hilafilcon B and Omafilcon B across different incubation periods demonstrate dynamic interactions between material properties and bacterial colonization, as illustrated in Fig. 2a. Initially, for non-incubated lenses, Hilafilcon B exhibited slightly higher bacterial adhesion (88.81%) compared to Omafilcon B (84.12%), suggesting that the surface properties of Hilafilcon B may be more conducive to initial bacterial attachment. After one day of incubation, both materials showed a notable reduction in bacterial adhesion, with Omafilcon B exhibiting a more pronounced decrease (69.13%) compared to Hilafilcon B (81.89%). This reduction likely reflects challenges faced by bacteria during the early adaptation phase [32]. By the seventh day, bacterial adhesion increased significantly for both materials, peaking at 93.78% for Hilafilcon B and 82.22% for Omafilcon B, indicating successful bacterial colonization under these conditions.

Interestingly, at 14 days, Hilafilcon B displayed a modest decline in adhesion (89.71%), while Omafilcon B exhibited an increase (91.52%), surpassing Hilafilcon B for the first time. This reversal may be attributed to differences in material properties, such as surface hydrophilicity or roughness, which may favor sustained bacterial attachment on Omafilcon B over time as happening in other lenses [33]. Finally, by 28 days, both materials showed reductions in bacterial adhesion, with Omafilcon B demonstrating the lowest adhesion level (64.38%) compared to Hilafilcon B (83.88%). This late-stage decline may result from nutrient depletion, bacterial competition, or the detachment of mature biofilms.

Similarly, the data comparing bacterial adhesion between cosmetic Hilafilcon B and Omafilcon B lenses across incubation periods, as presented in Fig. 2b, highlights important trends. For nonincubated lenses, Omafilcon B supported higher bacterial adhesion (101.09%) compared to Hilafilcon B (92.55%), indicating that Omafilcon B may initially provide a more favorable environment for bacterial attachment. After one day of incubation, both materials showed a decrement in bacterial adhesion, with Hilafilcon B (70.99%) and Omafilcon B (80.96%) reflecting reductions likely associated with the bacterial adaptation phase and pigments [34]. By the seventh day, adhesion increased again for both materials, with Omafilcon B (89.99%) maintaining higher colonization levels compared to Hilafilcon B (81.42%). This pattern suggests that both materials support bacterial regrowth under prolonged incubation conditions [35]. At 14 days, bacterial adhesion stabilized, with a slight decrease observed for Omafilcon B (81.83%) and Hilafilcon B (76.87%), possibly due to resource limitations or biofilm maturation. Notably, by 28 days, Hilafilcon B exhibited a resurgence in bacterial adhesion (95.43%), surpassing Omafilcon B (87.91%), which showed a modest decline. These results indicate that while Omafilcon B initially supports higher bacterial adhesion, Hilafilcon B becomes more susceptible over time, particularly with extended wear. These findings underscore the need for improved lens materials and robust hygiene practices to mitigate long-term bacterial colonization and associated risks.

3.3. S. aureus colonization

The data showcases changes in the colony-forming unit (CFU) of *Staphylococcus aureus* across incubation periods on both medical lenses as shown in Fig. 3a, and cosmetic as shown in Fig. 3b made from Hilafilcon B and Omafilcon B, with each experiment repeated three times and variability restricted to $\pm 5\%$, ensuring consistency and reliability. For non-incubated lenses, *S. aureus* adhesion starts at relatively similar levels for all materials, with Cosmetic-Omafilcon B and Medical-Omafilcon



Medical Hilafilcon B-Bacterial Adhesion Medical Omafilcon B-Bacterial Adhesion



Cosmetic Hilafilcon B-Bacterial Adhesion Cosmetic Omafilcon B-Bacterial Adhesion

Fig. 2. Bacterial Adhesion on contact lenses of different types (a. medical and b. cosmetic) and materials (Hilafilcon B and Omafilcon B) across incubation periods.

B exhibiting slightly higher CFU values (102.19%) compared to Cosmetic-Hilafilcon B and Medical-Hilafilcon B (101.17%). After one day of incubation, all lenses show a notable reduction in CFU, with the lowest adhesion observed for Hilafilcon B (77.66%) in both cosmetic and medical variants, while

Omafilcon B maintains slightly higher CFU values (80.14%).

By 7 days, the CFU values increase for all lenses, reflecting bacterial regrowth and adaptation, with Cosmetic-Hilafilcon B and Medical-Hilafilcon B exhibiting higher values (100.46%) compared to their



Fig. 3. Changes in CFU of S. aureus across incubation periods on: a. medical lenses and (b) cosmetic lenses made from Hilafilcon B and Omafilcon B. Each experiment was repeated three times with variability restricted to $\pm 5\%$, ensuring consistency and reliability of the results.

Omafilcon B counterparts (96.12%). At 14 days, the CFU values for Hilafilcon B remain slightly higher (101.53%) compared to Omafilcon B (97.81%), suggesting that Omafilcon B may exhibit slightly better resistance to bacterial colonization over prolonged periods. However, by 28 days, all materials exhibit increased CFU levels nearing their original values, with Cosmetic-Omafilcon B (102.96%) and Medical-Omafilcon B (106.16%) showing marginally higher bacterial adhesion compared to Hilafilcon B variants.

The trends observed indicate consistent bacterial colonization behaviors across both medical and cosmetic lenses, with Omafilcon B materials demonstrating marginally lower CFU levels at intermediate periods but slightly higher adhesion by the end of the incubation period. The small variability observed $(\pm 5\%)$ further supports the reliability of the experimental results. These findings underscore the need for enhanced cleaning regimens, especially for extended wear, and material innovations to mitigate biofilm formation on contact lenses.

The polymers used in contact lenses play a critical role in bacterial adhesion, biofilm formation, and their interaction with the tear film. These relationships are influenced by the material properties of the lenses, including hydrophilicity, surface roughness, and charge, as well as the biochemical composition of the tear film.

Contact lenses are typically made from hydrogel or silicone hydrogel polymers, each with unique characteristics that influence bacterial behavior. Hydrophilic polymers, such as Omafilcon B, form a water layer on their surface, creating a barrier that reduces bacterial adhesion. In contrast, materials like Hilafilcon B, with moderate water content and hydrophilicity, may support slightly higher bacterial adhesion. Surface roughness also plays a role; smoother surfaces minimize bacterial attachment, while rougher surfaces create niches that facilitate colonization. Additionally, the electrostatic properties of the polymer influence bacterial interactions, with neutral or negatively charged surfaces generally resisting adhesion more effectively than positively charged surfaces.

The tear film, a multi-layered fluid covering the eye, further modulates bacterial activity on contact lenses. Tear film components such as proteins (e.g., lysozyme, lactoferrin) and lipids adsorb onto the lens surface during wear, forming a conditioning layer that can either inhibit or promote bacterial adhesion. For example, hydrogels like Omafilcon B are more prone to protein deposition, which can act as a substrate for bacterial growth and biofilm formation, while silicone-based polymers may adsorb more lipids, altering the surface properties and potentially impacting bacterial colonization. Tear film pH, osmolarity, and the presence of antimicrobial proteins also influence the physiological state of bacteria on the lens surface.

Bacterial adhesion and biofilm formation are directly impacted by the interplay between lens polymers and the tear film. Materials that resist protein and lipid deposition tend to inhibit bacterial colonization, while those prone to these deposits can enhance bacterial growth. The tear film's antimicrobial properties, such as lysozyme activity, may be diminished by excessive protein or lipid accumulation on the lens surface, reducing its ability to combat microbial colonization. Furthermore, the tear film provides essential nutrients like glucose and amino acids, which can support bacterial growth on lenses, especially under prolonged wear conditions.

These interactions highlight the importance of selecting lens materials that resist bacterial adhesion and maintain tear film stability. Lenses with smooth surfaces, low protein-binding properties, and appropriate hydrophilicity can reduce bacterial colonization risks. Additionally, proper cleaning and disinfection are crucial for removing tear film deposits and minimizing bacterial growth. Understanding the interplay between lens polymers, bacterial actions, and tear film interactions underscores the need for continuous innovation in contact lens materials to improve ocular health and prevent infections.

4. Conclusion

Biofilm growth trends showed that lenses with ionic, high-water-content materials (Group IV) exhibited higher susceptibility to microbial colonization over time, particularly in cosmetic variants. Bacterial adhesion studies revealed dynamic interactions between S. aureus and lens surfaces, with Omafilcon B demonstrating slightly higher resistance in intermediate stages compared to Hilafilcon B. The Kirby Bauer method confirmed the effectiveness of antibacterial contact lenses in reducing microbial colonization, with measurable zones of inhibition supporting their efficacy. The ionic surface of Hilafilcon B facilitates electrostatic interactions between the negatively charged bacterial cells (such as S. aureus) and the positively charged groups on the lens surface, promoting stronger bacterial adhesion. Additionally, cosmetic Hilafilcon B lenses often undergo surface modifications, including pigmentation layers, which may increase surface roughness and create microenvironments that further support bacterial attachment and biofilm formation. These combined factors-ionic charge, high water content, and surface modifications-contribute to the increased susceptibility of Hilafilcon B cosmetic lenses to microbial colonization.

The findings underscore the critical role of lens material in determining bacterial interactions and highlight the importance of enhanced hygiene protocols and material innovation to mitigate microbial risks. The results emphasize the need for targeted interventions, including optimized lens cleaning regimens and the development of advanced lens materials with antimicrobial properties, to ensure user safety and reduce the potential for ocular infections. This research contributes valuable insights into the performance of contact lenses under simulated ocular conditions, providing a foundation for future advancements in contact lens design and care.

Appendix A

The questionnaire collected data from Iraqi contact lens users on lens types, usage habits, wear duration, and eye health issues, guiding material selection and ensuring real-world relevance in the study. The complete responses of 779 participants are provided in the supplementary file "S1".

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval

Not applicable.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Lina M. Shaker and Wan Nor Roslam Wan Isahak contributed equally to this study. Lina M. Shaker was responsible for the study conception, experimental design, and data analysis. Wan Nor Roslam Wan Isahak conducted the experiments, contributed to data interpretation, and drafted the initial manuscript. Both authors critically revised the manuscript and approved the final version.

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