Kadhum et al, Babcock Univ. Med. J.2026 9(1):258-264
https://doi.org/10.38029/babcockuniv.med.j..v9i1.1342
eISSN: 2756-4657

RESEARCH ARTICLE OPEN ACCESS

# Inhibitory effect of chlorhexidine on biofilm formation by prosthetic surface pathogens: An in vitro study

Kadhum FI[1], AlKhayyat FNMT[2], Jamal HM[3]

[1]Department of Dentistry, Al-Hikma University College, Baghdad, Iraq
[2]Department of Prosthodontics, College of Dentistry, Mustansiriyah University, Baghdad, Iraq
[3]College of Dentistry, Al-Bayan University, Iraq

*Submitted: 16th November 2025*
*Accepted: 21st January 2026*
*Published: 31st March 2026*
ID: Orcid ID

**Abstract**

**Objective:** The research investigated how 0.12% chlorhexidine affects the initial growth of single-species biofilms, which include typical prosthetic surface bacteria that colonise different types of prosthetic materials.

**Methods:** Discs of titanium, zirconia, cobalt-chromium alloy, PMMA, and PEEK were inoculated with five common prosthetic pathogens (S. aureus, S. mutans, E. faecalis, C. albicans, P. aeruginosa). After 24-hour biofilm formation, specimens were treated with 0.12% CHX for 60 seconds. Biofilm biomass was assessed by crystal violet staining and CFU enumeration.

**Results:** The tested materials showed decreased early biofilm development after Chlorhexidine exposure at different levels, which depended on material surfaces and bacterial species. The surface materials of titanium and zirconia showed the most significant decrease in biofilm biomass, but PMMA and PEEK surfaces maintained more biofilm than the other materials. The susceptibility tests showed that S. aureus and S. mutans became more responsive to chlorhexidine treatment, yet C. albicans and P. aeruginosa developed stronger resistance against the treatment.

**Conclusion:** Chlorhexidine shows different levels of biofilm inhibition on prosthetic implant surfaces when tested under laboratory conditions based on both the material used and the species being studied. The research results confirm that chlorhexidine should be used as an additional measure for prosthesis cleaning, but the study shows that choosing the right materials remains essential to prevent biofilm-related problems. The research needs additional investigations, which should include surface analysis and multiple species biofilm experiments.

**Keywords:** Chlorhexidine, biofilm, titanium, zirconia, PMMA

## Plain English Summary

In this laboratory experiment, the ability of the popular antiseptic chlorhexidine (in a 0.12% liquid form) to prevent the initial proliferation of bacterial and fungal films (so-called biofilms) on various materials used to manufacture dental implants and dentures was tested. The test materials were titanium, zirconia, cobalt-chromium alloy, PMMA (plastic), and PEEK (high-performance plastic). Scientists cultured biofilms of five popular germs on the materials and treated them with chlorhexidine in one minute.

The results showed that chlorhexidine reduced the biofilm on all materials, but some worked better than others. Titanium and zirconia had the least biofilm left after treatment. The plastic materials (PMMA and PEEK) held onto more biofilm, making them harder to clean. The effectiveness also depended on the type

Correspondence:
Jamal Hamsa M
College of Dentistry
Al Bayan University
Iraq
Hamsa.jamal@albayan.edu.iq

of germ: bacteria like Staphylococcus aureus were easily reduced, but the fungus Candida albicans and the bacterium Pseudomonas aeruginosa were more resistant, especially on the plastic surfaces.
The study concludes that while chlorhexidine is a helpful disinfectant, its success depends heavily on both the prosthetic material and the specific germs present. For non-metal materials like dentures, chlorhexidine should be used alongside thorough mechanical cleaning (like brushing) for the best results. This highlights the need to choose implant materials carefully and tailor cleaning routines based on the material used.

## Introduction

The materials used for prosthetic implants serve modern dental patients, but their long-term clinical performance remains at risk due to microbial growth, leading to biofilm development. Biofilms develop through microbial community organisation, which produces a protective extracellular matrix to defend against antimicrobial agents and host defence mechanisms. The formation of biofilms on prosthetic materials leads to the development of peri-implant diseases and prosthesis-associated infections, which makes early microbial adhesion control essential for prevention (1).

The physicochemical properties of prosthetic materials, which include surface roughness, surface free energy, and chemical composition, determine how microorganisms first attach to surfaces and how biofilms develop. The surface properties of titanium and zirconia and PMMA, cobalt–chromium alloys and polyetheretherketone (PEEK) differ significantly from each other, which could affect their ability to support microbial growth (2, 3). Research must continue to study material-dependent features which impact early biofilm formation because it will help develop improved materials for extended prosthetic device durability. Chlorhexidine (CHX) is regarded as the gold standard chemical plaque control agent in dentistry due to its broad-spectrum antimicrobial activity and unique substantivity, which enables prolonged retention on oral surfaces. While CHX has been extensively studied for plaque control on natural teeth and some implant surfaces, its specific efficacy in preventing early biofilm formation on diverse prosthetic biomaterials, including modern polymers like PEEK, has not been systematically compared under controlled laboratory conditions. (4, 5). Its substantivity allows prolonged adsorption to surfaces, providing extended antimicrobial activity (6). Standardised laboratory tests have not fully evaluated how chlorhexidine prevents biofilm formation on different prosthetic materials, although it remains a widely used medical treatment (7, 8).

The following microorganisms, which cause prosthetic and peri-implant infections, show different patterns of adhesion and biofilm development according to research findings: Staphylococcus aureus (9), Streptococcus mutans (10), Candida albicans (11, 12, 13) and Pseudomonas aeruginosa and Enterococcus faecalis (14, 15). The different ways these bacteria react to antiseptic agents create additional challenges for infection control because they need to handle different types of prosthetic materials. The evaluation of chlorhexidine effectiveness against these important dental pathogens, which infect different dental materials, requires assessment to develop proper preventive and maintenance strategies for prosthetic dentistry (16, 17). However, despite its widespread clinical use, comparative data on the efficacy of CHX against early biofilm formation across the range of contemporary prosthetic materials, particularly under standardised in vitro conditions, remain limited. Most existing studies have focused on individual materials or selected pathogens, leaving a need for systematic evaluation of how material properties modulate CHX-mediated biofilm inhibition.

Based on the documented influence of material surface properties on microbial adhesion and the variable susceptibility of different microorganisms to antiseptics, we hypothesised that the efficacy of chlorhexidine against early biofilm formation would differ significantly across prosthetic materials and microbial species. Accordingly, this in vitro study was designed to systematically evaluate and compare the inhibitory effect of 0.12% chlorhexidine on early monospecies biofilm formation by five key prosthetic pathogens across five commonly used prosthetic implant materials. The research investigates which materials affect biofilm development after chlorhexidine treatment at predetermined concentrations to identify factors which affect prosthesis cleaning and antiseptic performance (4, 5).

## Materials and Methods

*Study Design*

This study was conducted as a controlled in vitro experimental investigation to evaluate the inhibitory effect of chlorhexidine (CHX) on early monospecies biofilm formation on different prosthetic implant materials. The research used laboratory tests to study biofilm development by applying chlorhexidine for a short time to

specimens while keeping untreated control samples.

*Prosthetic Materials*

The following five prosthetic materials are frequently employed in dental practice: titanium and zirconia, cobalt–chromium alloy, polymethylmethacrylate (PMMA) and polyetheretherketone (PEEK). The laboratory followed established procedures to create disc-shaped specimens, which measured 10 mm in diameter and 2 mm in thickness for each material. Six discs were prepared for each material-microorganism combination (n = 6 per group), comprising both technical and biological replicates to ensure methodological robustness. The specimens received mechanical polishing through a controlled method to smooth their surfaces before undergoing ultrasonic cleaning with distilled water and sterilisation procedures.

*Microorganisms and Culture Conditions*

The research used Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, Candida albicans and Pseudomonas aeruginosa to study biofilm development because these microorganisms frequently infect prosthetic surfaces. The bacterial species and C. albicans cultures underwent aerobic growth in Brain Heart Infusion (BHI) broth for bacterial species and Sabouraud dextrose broth for C. albicans. The researchers adjusted Overnight cultures to reach a 0.5 McFarland standard before creating a working inoculum for biofilm development.

*Biofilm Formation Protocol*

Each sterile disc was placed individually into a well of a 24-well tissue culture plate and inoculated with microbial suspension. The plates needed 24 hours of static incubation at 37 °C to establish their first biofilm structures. The researchers incubated the discs before they performed two sterile phosphate-buffered saline (PBS) washes to remove all non-adherent cells.

*Chlorhexidine Exposure*

A 0.12% chlorhexidine gluconate solution was prepared from a commercial stock (Chlorhexidine Gluconate 20% Solution, Sigma-Aldrich, St. Louis, MO, USA) by dilution with sterile distilled water to match the concentration commonly used in clinical mouthrinses. Test specimens were immersed in 0.12% chlorhexidine gluconate solution for 60 s at room temperature. The selected concentration and exposure time followed standard clinical mouthrinse procedures, which dentists use in their practice. The control specimens received identical treatment, but they were placed in a sterile PBS solution, which replaced the chlorhexidine solution. While a specific neutralising agent was not employed, extensive washing with sterile PBS (three cycles of 1-minute washes with agitation) was performed to minimise residual CHX carryover, consistent with methods described in similar in vitro biofilm studies.

The researchers used sterile PBS to rinse specimens right after chlorhexidine exposure because they wanted to reduce the amount of antiseptic that could interfere with microbiological tests.

*Biofilm Quantification*

*Crystal Violet Assay*

*The crystal violet staining was used to quantify the biofilm biomass. The researchers utilized crystal violet solution of 0.1 percent on the discs and left them to stay in the solution of crystal violet 15 minutes after which they soft rinsed the discs to eliminate any leftover dye. The stained bound needed 95 percent ethanol to dissolve, and then the microplate reader could read and record the absorbance at 570 nm. The research team offered its findings in the form of measurements that indicated the extent to which biofilm biomass reduced as compared to control samples that had no treatment.*

*Colony-Forming Unit Enumeration*

*The viable cell quantification process required mechanical agitation to remove biofilms on disc surfaces to sterile PBS. The laboratory personnel followed the procedure of serial dilution then proceeded to plate the samples on the appropriate agar media after which they incubated the samples at 37 degC. Colony-forming units (CFU) were used as indicators of measuring the amount of viable biofilm-associated microorganisms that resisted the chlorhexidine treatment.*

*Statistical Analysis*

Given the exploratory nature of this study and the focus on observing material- and microorganism-dependent trends rather than testing predefined hypotheses, data were analysed descriptively. Biofilm reduction percentages were calculated relative to untreated controls for each material-microorganism combination. The consistency between crystal violet absorbance and CFU enumeration results was assessed qualitatively rather than through inferential statistical testing, as the primary aim was to identify patterns of response rather than to establish statistical significance.

## Results

*Overall Effect of Chlorhexidine on Biofilm Formation*

The tested prosthetic materials developed smaller early monospecies biofilms after being treated with 0.12% chlorhexidine compared to control specimens that received no treatment. This inhibitory effect was confirmed using two complementary methods, which included crystal violet staining and colony-forming unit (CFU) enumeration to show reduced total biofilm biomass and number of living microorganisms that adhered to surfaces.

The extent of biofilm reduction depended on both the prosthetic material type and the microbial species, which showed different responses to chlorhexidine treatment.

*Effect of Chlorhexidine According to Prosthetic Material*

The extent of biofilm reduction differed among the tested prosthetic substrates (Table 1). The biofilm formation process achieved its minimum point when researchers treated titanium and zirconia discs with chlorhexidine. The tested materials demonstrated reduced biofilm residue for all studied microorganisms when researchers compared them to unprocessed control samples. The biofilm biomass and viable cell numbers on Cobalt–chromium alloy surfaces showed an average response to chlorhexidine treatment, which resulted in significant decreases. The polymer-based materials PMMA and PEEK showed better resistance to biofilm degradation because they maintained significant amounts of biofilm residue after exposure. Although biofilm formation on these materials was reduced relative to controls, the magnitude of inhibition was lower than that observed on metallic and ceramic surfaces.

**Table 1: Relative percentage reduction of early monospecies biofilm formation on different prosthetic materials following exposure to 0.12% chlorhexidine**

| Material | *S. aureus* | *S. mutans* | *E. faecalis* | *C. albicans* | *P. aeruginosa* |
|---|---|---|---|---|---|
| Titanium | 70% | 68% | 50% | 35% | 30% |
| Zirconia | 68% | 65% | 48% | 33% | 28% |
| Co–Cr alloy | 50% | 48% | 35% | 25% | 20% |
| PMMA | 35% | 32% | 25% | 15% | 10% |
| PEEK | 33% | 30% | 22% | 12% | 8% |

Values represent relative percentage reductions in biofilm formation following a single 60-s exposure to 0.12% chlorhexidine compared with untreated control specimens. Percentages were derived from crystal violet absorbance measurements and supported by colony-forming unit enumeration. Data are presented descriptively to illustrate material- and microorganism-dependent trends in early biofilm inhibition

*Effect of Chlorhexidine According to Microbial* Species

The susceptibility of the microorganisms to chlorhexidine also differed among the test organisms (Table 1). The highest relative decrease in biofilm formation was displayed in Staphylococcus aureus and Streptococcus mutans of all the material types. Enterococcus faecalis exhibited a moderate response of biofilm formation reduction after treatment.

On the other hand, Candida albicans and Pseudomonas aeruginosa showed relatively increased residual biofilm formations following exposure to chlorhexidine, especially on PMMA and PEEK. This means that these organisms are less likely to succumb to short term chlorhexidine-treatment under the conditions applied in the laboratory.

*Comparison Between Biofilm Assessment Methods*

Results obtained from crystal violet staining were consistent with those derived from CFU enumeration. Materials and microbial species that exhibited lower crystal violet absorbance values also showed corresponding reductions in viable cell counts. This agreement suggests that reductions in biofilm biomass were accompanied by parallel decreases in viable adherent microorganisms.

*Summary of Observed Trends*

Chlorhexidine decreased the early biofilm formation on all the tried prosthetic materials.

The extent of inhibition depended on the type of material with titanium and zirconia having the least amount of residual biofilm.

Materials that were made of polymer (PMMA and PEEK) had increased residual biofilm levels after treatment.

Microbial susceptibility to chlorhexidine was species-dependent, with S. aureus and S. mutans being more responsive than C. albicans and P. aeruginosa.

Chlorhexidine decreased the early biofilm formation on all the tried prosthetic materials.
The extent of inhibition depended on the type of material with titanium and zirconia having the least amount of residual biofilm.
Materials that were made of polymer (PMMA and PEEK) had increased residual biofilm levels after treatment.

**Discussion**

The current in vitro experiment is able to show that even one, short exposure to 0.12% chlorhexidine (CHX) can considerably prevent the spontaneous early development of monospecies biofilms on various prosthetic materials. The most significant conclusion, though, is that the extent of such inhibition is not universal; instead it is in essence regulated by a dual interaction between the physicochemical characteristics of the prosthetic substrate, and the intrinsic vulnerability of the microbial species (1, 4, 5). This confirms our hypothesis and highlights the problem of complexity of biofilm control in the context of the present-day field of prosthetic dentistry where choice of a material and choice of antimicrobial approach should be taken as a pair.
The greatest reduction in biofilm was recorded on zirconia and titanium surfaces. This is in line with well-known principles of microbial adhesion, in which smoother, less porous and lower surface energy substances are known to support a reduced microbial adhesion as well as possibly, allow easier antimicrobial infiltration (2, 3). This enhanced performance of CHX on these substrates is probably due to a composite of three reasons: intrinsically reduced baseline biofilm formation and a topographical surface that does not provide covering adherent cells with the antiseptic agent. Conversely, the polymer-based materials,PMMA and PEEK, had considerably higher residual biofilm. This is explained by their generally greater surface roughness, porosity, and surface chemistry that enhances greater microbial adhesion and formation of protective niches that restrict the contact and activity of topical antiseptics such as CHX (7, 8). Such a material-dependent efficacy gradient- between high-performance ceramics and metals and more retentive polymers- gives a reasonable explanation of the evidence-based material choice in patients who are under a high risk of biofilm-mediated complications.
The experiment also found out that there are stark species-dependent susceptibility differences to CHX. The most sensitive ones were staphylococcal aureus and Streptococcus mutans (Gram-positive bacteria), which is also in line with the major mechanism of action of CHX, which includes rupturing the cytoplasmic membrane and spewing the cytoplasmic contents (4, 5). The intermediate efficacy against Enterococcus faecalis is impressive, because this organism is commonly linked to the persistent peri-implant and endodontic infections, and is commonly very tolerant of antiseptics (3). More importantly, Candida albicans and Pseudomonas aeruginosa were the most resilient. In the case of C. albicans, this is attributed to the complicated structure of fungal biofilms, which have a dense extracellular matrix and also due to the drug-persister phenotypes which are notoriously hard to eliminate with short-term antiseptic exposure (9, 11, 12, 13). P. aeruginosa also harbors a powerful set of resistance mechanisms, such as efflux pumps, inducible stress responses, and also the generation of a powerful extracellular polymeric substance (EPS) rich in alginate which happens to be a diffusion barrier against antimicrobials
(14, 15). The overlapping between the increased residual biofilm of the two species on PMMA and PEEK already-retentive surfaces is a crucial point of observation, which indicates a high-risk material-pathogen interaction.
The fact that the results of crystal violet staining are strongly correlated with the results of viability indicators in terms of CFU enumeration reinforces the validity of our results (6, 7). The same agreement suggests that a decrease in the total biofilm biomass related directly to a decrease in viable attaching microorganisms, which validates that the activity of CHX in these conditions was bacteria/fungicidal and not just a disruptive action to the biofilm matrix.

Clinically, these findings confirm the usefulness of CHX as a secondary chemical agent in the use of hygiene procedures in the care of prostheses. But they also do define its limitations pretty clearly. The information indicates that though CHX can be very efficient in regular cleaning of titanium and zirconia parts, it might not be effective enough in the decontamination of polymeric denture bases (PMMA) or PEEK frameworks, particularly in the presence of C. albicans or P. aeruginosa. This requires a paradigm wherein the hygiene of prosthesis is material specific. In the case of polymeric materials, CHX ought to. be viewed as a supplement to, not a replacement for, rigorous mechanical cleaning (e.g., brushing) and, in some cases, may need to be combined with other antimicrobial strategies (16, 17)

**Study limitations**

The findings of this study should be regarded within its scope of methodology. Although in vitro monospecies biofilm model is superb in controlled comparison, it lacks the complexity that is represented by the in vivo oral environment that comprises multispecies biofilm, salivary pellicle formation, host immune factors, and fluid dynamics (1, 2). The concentration and exposure time are single; clinically relevant, but they are not investigating the potential of longer applications and alternative CHX preparations. Moreover, the descriptive analysis mode, adopted to determine the early trends, is insufficient to make some conclusive statistical statements regarding relative effectiveness and should be followed by further confirmatory investigations based on hypothesis-driven inferential statistics.

**Conclusion**

In conclusion, this study provides a detailed in vitro map of how chlorhexidine efficacy against early biofilm formation is co-determined by prosthetic material and microbial species. It reinforces the "gold standard" status of CHX while offering a nuanced view that should inform clinical practice. Effective long-term prosthetic maintenance will depend on integrating such chemical strategies with thoughtful material selection and complementary mechanical hygiene methods. Future research should focus on validating these findings in multispecies biofilm models, investigating the efficacy of CHX against mature biofilms, and exploring surface modification techniques to enhance the antifouling properties of polymeric prosthetic materials.

Future investigations should validate these findings using multispecies biofilm models and clinically relevant exposure protocols to optimise evidence-based preventive strategies for prosthetic maintenance.

**Declarations**

*Ethics Approval and Consent to Participate*

Given that this research was in fact completely done in the laboratory and did not include any persons or personal data of any kind, all work was performed as per the relevant institutional and national ethical standards for laboratory-based (non-human subject) research. Consequently, the research was exempt from ethics approval involving humans and informed consent.

*Consent for publication*

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

*Data Availability*

Data for this work is available.

*Competing Interests*

None

*Funding*

None.

*Author Contributions*

KFI: Conceptualisation, Writing – Review & Editing, Supervision and Project Administration.
JHM: Investigation, Formal Analysis, Writing – Original Draft Preparation
KFI and JHM: Methodology

*Acknowledgments*

None.

**References**

1. Subramani K, Jung RE, Molenberg A, Hammerle CH. Biofilm on dental implants: a review of the literature. Int J Oral Maxillofac Implants. 2009 Jul-Aug;24(4):616-26.
2. Quirynen M, Bollen CM. The influence of surface roughness and surface-free energy on supra-and subgingival plaque formation in man: A review of the literature. Journal of clinical periodontology. 1995 Jan;22(1):1-4. https://doi.org/10.1111/j.1600-051X.1995.tb01765.x
3. Jones CG. Chlorhexidine: is it still the gold standard?. Periodontology 2000. 1997 Oct;15:55-62. https://doi.org/10.1111/j.1600-0757.1997.tb00105.x
4. Van Strydonck DA, Slot DE, Van der Velden U, Van der Weijden F. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: a systematic review. Journal of clinical periodontology. 2012 Nov;39(11):1042-55. https://doi.org/10.1111/j.1600-051X.2012.01883.x
5. Gosau M, Hahnel S, Schwarz F, Gerlach T, Reichert TE, Bürgers R. Effect of six different peri-implantitis disinfection methods on in vivo human oral biofilm. Clinical oral implants research. 2010 Aug;21(8):866-72. https://doi.org/10.1111/j.1600-0501.2009.01908.x

6. Persson GR, Renvert S. Cluster of bacteria associated with peri-implantitis. Clinical implant dentistry and related research. 2014 Dec;16(6):783-93. https://doi.org/10.1111/cid.12052
7. Müller HD, Eick S, Moritz A, Lussi A, Gruber R. Cytotoxicity and antimicrobial activity of oral rinses in vitro. BioMed research international. 2017;2017(1):4019723. https://doi.org/10.1155/2017/4019723
8. Roehling S, Astasov-Frauenhoffer M, Hauser-Gerspach I, Braissant O, Woelfler H, Waltimo T, Kniha H, Gahlert M. In vitro biofilm formation on titanium and zirconia implant surfaces. Journal of periodontology. 2017 Mar;88(3):298-307. https://doi.org/10.1902/jop.2016.160245
9. Ramage G, Martínez JP, López-Ribot JL. Candida biofilms on implanted biomaterials: a clinically significant problem. FEMS yeast research. 2006 Nov 1;6(7):979-86. https://doi.org/10.1111/j.1567-1364.2006.00117.x
10. Busscher HJ, Rinastiti M, Siswomihardjo W, Van der Mei HC. Biofilm formation on dental restorative and implant materials. Journal of dental research. 2010 Jul;89(7):657-65. https://doi.org/10.1177/0022034510368644
11. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nature Reviews Disease Primers. 2018 May 11;4(1):1-20. https://doi.org/10.1038/nrdp.2018.26
12. Bürgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, Gosau M. In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clinical oral implants research. 2010 Feb;21(2):156-64. https://doi.org/10.1111/j.1600-0501.2009.01815.x
13. Hahnel S, Wieser A, Lang R, Rosentritt M. Biofilm formation on the surface of modern implant abutment materials. Clinical oral implants research. 2015 Nov;26(11):1297-301. https://doi.org/10.1111/clr.12454
14. Busscher HJ, Van Der Mei HC, Subbiahdoss G, Jutte PC, Van Den Dungen JJ, Zaat SA, Schultz MJ, Grainger DW. Biomaterial-associated infection: locating the finish line in the race for the surface. Science translational medicine. 2012 Sep 26;4(153):153rv10-. https://doi.org/10.1126/scitranslmed.3004528
15. Subbiahdoss G, Grijpma DW, Van der Mei HC, Busscher HJ, Kuijer R. Microbial biofilm growth versus tissue integration on biomaterials with different wettabilities and a polymer-brush coating. Journal of Biomedical Materials Research Part A. 2010 Aug;94(2):533-8. https://doi.org/10.1002/jbm.a.32731
16. De Angelis F, D'Ercole S, Di Giulio M, Vadini M, Biferi V, Buonvivere M, Vanini L, Cellini L, Di Lodovico S, D'Arcangelo C. In vitro evaluation of Candida albicans adhesion on heat-cured resin-based dental composites. Materials. 2023 Aug 25;16(17):5818. https://doi.org/10.3390/ma16175818
17. Shafiei F, Jowkar Z, Eslamipanah S, Mohammadi-Bassir M, Doozandeh M, Kowkabi M. Comparative evaluation of chlorhexidine and benzalkonium chloride pretreatments on bond strength durability of immediate dentin sealing with universal adhesive: an in vitro study. BMC Oral Health. 2025 Mar 15;25(1):390. https://doi.org/10.1186/s12903-025-05745-5