ORIGINAL ARTICLE
DOI: 10.5152/EssentDent.2026.25056

Evaluation of Bacterial Growth on Different Dental Materials

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Cite this article as: Ateş G. Evaluation of bacterial growth on different dental materials. *Essent Dent.* 2026, 5, 0056, doi:10.5152/EssentDent.2026.25056.

Abstract

Background: This study aimed to evaluate the effects of rinsing and disinfecting dental materials, including acrylic resin denture bases, shellac, ceramics, and metal alloys, that were contaminated with saliva before being placed in a dental cast.

Methods: Three hundred fifty-two (N = 352) cylindrical samples of 4 mm in height and 10 mm in diameter were prepared, 88 of each dental material (acrylic resin denture base, shellac, ceramic, and metal alloy). The samples were divided into 4 groups, each containing 22 samples. All samples were contaminated with 25 µL of unstimulated saliva and were treated differently: some were rinsed in cold running tap water, others were scrubbed with soap and cold running tap water, and still others were soaked in 2% glutaraldehyde. As controls, some samples were contaminated with saliva and left untreated (n = 22). Sheep blood agar plates were inoculated and incubated at 37°C for 72 hours. The colonies of bacteria were counted after 24 hours. Data were analyzed using ANOVA ($P < .05$), and the Kruskal–Wallis test was used for specific comparisons.

Results: This study demonstrated that rinsing saliva-contaminated dental materials for 20 seconds under cold running tap water significantly reduced bacterial contamination ($P < .05$). Similarly, scrubbing with antiseptic soap or immersion in 2% glutaraldehyde solution for 20 seconds effectively reduced bacterial contamination.

Conclusion: The findings of this study demonstrated the importance of rinsing or disinfecting dental materials after use in the patient's mouth and before placement in a dental cast to prevent cross-infection. These results can significantly contribute to increasing infection control measures in dental clinics and laboratories.

Keywords: Bacterial growth, cross-infection, dental materials, disinfection, saliva contamination

What is already known on this topic?

- *During prosthesis construction, impression materials and dental casts are usually disinfected to control cross-infections. However, disinfecting only the impressions taken from the mouth and dental cast is insufficient, especially in prosthesis fabrication, which can last for several sessions. This is because prosthetic materials are contaminated with saliva during each session in which the prosthesis is tried in the mouth.*
- *In addition, more than 1 disinfection of the cast causes the deterioration of the plaster surface. This deterioration of the surface also causes deterioration of prosthesis surfaces.*

What does this study add on this topic?

- *This study shows that disinfecting dental materials used in prosthesis construction, especially after contamination with saliva at each session, is highly effective.*
- *Furthermore, disinfection of oral impressions and dental casts alone is insufficient to prevent reinfection of prosthetic materials and thus cross-infection.*

INTRODUCTION

Recently, awareness of infectious diseases and their transmission methods has increased. This increased awareness has also influenced attitudes toward cross-infection control in dental clinics and laboratories. Infection control, the cornerstone of dentistry, is crucial because of the high transmission potential of many microorganisms encountered during dental treatment. The implementation of a standard disinfection process for cross-infection control is also crucial for ensuring patient safety. Infection prevention and control involves a series of evidence-based practices designed to protect patients and healthcare workers from preventable infections. It is the responsibility of healthcare professionals to ensure that the standards set here are applied correctly.[1] Awareness

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Received: May 15, 2025
Revision Requested: June 20, 2025
Last Revision Received: August 31, 2025
Accepted: October 13, 2025
Publication date: March 25, 2026

and interest in infection control have become even more important, especially after the COVID-19 pandemic. Over 700 bacteria, fungi, and viruses have been detected in oral fluids. These microorganisms can potentially cause dental and periodontal infections[2] Oral infections also lead to many chronic systemic diseases.[3]

Cross-infection is defined as the transmission of infectious pathogens from 1 healthcare personnel to another or from 1 patient to another within a healthcare facility.[4]The potential for cross-contamination is intimately linked to the exchange of information and physical contact between dental professionals and dental laboratories.[5]

Owing to the nature of prosthetic treatment, preventing cross-contamination is a significant challenge in this field. It is imperative to note that oral fluids, including blood and saliva, have the potential to contain viruses or bacteria that can cause various infectious diseases.[6]These diseases may include, but are not limited to, colds, herpes simplex virus, varicella-zoster virus, hepatitis B and C, pneumonia, tuberculosis, *Mycobacterium* spp., multidrug-resistant bacteria, and human immunodeficiency virus.[7-9]

In addition to the infectious diseases, dentists, dental clinics, and laboratory workers are susceptible to contracting the novel coronavirus, SARS-CoV-2, through exposure to saliva.[10]

The infection can be transmitted through direct contact via blood, saliva, the nose, or the eyes, or aerosol particles, or indirectly through contaminated instruments, equipment, and dental materials during dental procedures.[11-17]

The fabrication of prosthetic restorations involves multiple sessions, during which the dental cast can become contaminated repeatedly. This occurs when materials such as acrylic resin denture bases, shellac, metal alloys, and ceramics are placed in a dental cast without being cleaned or disinfected after use in the mouth. Research has shown that dental impressions and casts may harbor opportunistic pathogens.[18,19]

Furthermore, a previous study revealed that dental impressions, dentures, crowns, and dental wax occlusal templates received from dental laboratories contained 67% harmful bacteria.[20] Another study confirmed that microorganisms may be transferred from dental impressions to dental casts.[21]Previous research has found that up to 88% of laboratory work received and sent to dental clinics was contaminated with a mixture of flora and microorganisms.[22]

Various dental materials may contaminate dental casts. Recent studies show dentists are at high risk of contracting and transmitting infectious diseases during treatments.[23] The literature contains limited data on heat-cured resin, although the effectiveness of chemically activated acrylic resin disinfection has been previously evaluated.[24]

In prosthodontics, materials that may be contaminated with pathogenic bacteria are often transferred between dental laboratories and clinics. A standard dental procedure that presents a potential risk of cross-contamination is the making and pouring of impressions.[25,26]

Dental materials, such as acrylic resin denture bases, shellac, metal alloys, and ceramic, are placed in the mouth during clinical try-in appointments for removable and fixed prostheses. These materials may become recontaminated with saliva during the following processes: try-in, inter-occlusal record, wax rims, tooth alignment, final delivery of the prostheses, and repair of broken dentures. Dental casts and these prosthetic materials should be disinfected after each clinical and laboratory procedure.

Dentists, dental assistants, and dental laboratory staff should implement effective infection control procedures to prevent the transmission of diseases. The American Dental Association Council on Scientific Affairs provides standards for cleaning and disinfecting impressions, casts, and intraoral prostheses used in dental practice before they are sent to dental laboratories.[27,28]

The importance of disinfecting prosthetic materials in dental clinics and laboratories to prevent cross-contamination has been widely acknowledged. Nevertheless, no standard method has been established for achieving this goal. Identifying a simple, cost-effective, and non-damaging disinfecting method for prosthetic materials is essential.

This study aimed to evaluate the effect of rinsing or disinfecting dental materials contaminated with saliva, such as acrylic resin denture bases, shellac, ceramics, and metal alloys, on bacterial growth.

MATERIALS AND METHODS

Ethical approval was obtained from the Health Sciences Ethics Committee of Ankara Yıldırım Beyazıt University (meeting date: November 21, 2024; approval code: 09/982). This study used 4 different dental materials, which are listed below:

- Acrylic resin denture base: Stellon QC 20 (Dentsply Co)
- Shellac: Cavex (Holland BV)
- Ceramic material: Ceramco 3 Feldspathıc Porcelain (Dentsply)
- Ni-Cr metal alloy: Heraenium NA (Kulzer)

Three hundred fifty-two cylindrical samples (n=352) were prepared, each measuring 4 mm in height and 10 mm in diameter. There were 88 (n=88) samples of each type of dental material: acrylic resin denture base, shellac, ceramic, and nickel-chromium metal alloy. Feldspathic ceramic samples were sintered but not glazed to observe bacterial adhesion during dentin try-in the mouth. Acrylic resin and metal

Table 1. Dental Materials Used in This Study

Dental Materials	Contamination with Saliva	Rinsing in Cold Tap Water	Rinsing with Soap+Brushing	Soaking in Glutaraldehyde	Total
Acrylic resin denture base	22	22	22	22	88
Shellac	22	22	22	22	88
Ceramic	22	22	22	22	88
Nickel-chromium metal alloy	22	22	22	22	88

alloy samples were subjected to leveling and polishing processes. Besides, metal alloy samples were cleaned in an ultrasonic cleaner. Shellac samples were leveled but not polished. A volume of 5 mL of unstimulated saliva from 1 person was collected in a sterile, disposable saliva collector. To maintain standardization, each sample was immersed in 25 µL of saliva collected from the same person for 5 minutes. Subsequently, the 88 (n=88) samples from each dental material were divided into 4 subgroups of 22 (n=22) samples each, and 4 different treatments were applied to each subgroup (Table 1).

The first subgroup was contaminated with saliva and received no further treatment. After saliva contamination, the second subgroup was rinsed with cold running tap water for 20 seconds (Figure 1).

The third subgroup was scrubbed with a soft toothbrush for 20 seconds using antiseptic hand soap (Protex) and cold running tap water after saliva contamination (Figure 2). After saliva contamination, the fourth subgroup was immersed in 2% glutaraldehyde for 20 seconds.

After each sample was treated, it was placed in a sterile test tube (KIMAX Culture Tubes with Screw Cap, Nelson-Jameson, Texas) containing 2.5 mL of sterile phosphate-buffered saline (PBS) and allowed to sit for 20 seconds. Dilutions were performed to reduce the relative number of colony-forming units (CFUs) to facilitate counting and manage quantities more effectively.

The suspensions were agitated, and 25 µL aliquots were transferred to wells in a sterile microtiter plate containing 225 µL of sterile PBS, resulting in a final volume of 250 µL and a 10^{-3} dilution. After being mixed by pipette, a 25 µL aliquot was transferred to a second well containing 225 µL sterile PBS, creating a final dilution of 10^{-4}. To determine the bacterial count in the 10^{-4} dilution, 100 µL of the total solution volume of 250 µL was transferred to a sheep blood agar plate. A plate spreader was used to obtain a lawn of growth on the surface of the plate. Later, the total count of colonies per plate was calculated as CFUs × 2.5 × 10^{-4}. Blood agar plates were incubated at 37°C for 72 hours. The bacterial colonies were visually counted after 24 hours of incubation. Data were analyzed using ANOVA with a significance level of $P < .05$, and the Kruskal–Wallis test was used for specific comparisons.

RESULTS

All dental material samples that were not cleaned after contamination with saliva showed large bacterial colonies on the blood agar plates. On average, 259 colonies grew on acrylic resin, 240 on shellac, 330 on ceramic, and 329 on metal alloy samples that were not treated after being contaminated with saliva (Tables 2 and 3).

Acrylic resin denture base samples contaminated with saliva, without further treatment, were found to have a mean of 259 colonies at a 10^{-4} dilution. The acrylic resin samples contaminated with saliva and then rinsed with tap water had a

Figure 1. Rinsing with tap water.

Figure 2. Brushing with antiseptic soap and a toothbrush in tap water.

mean of 5.41 colonies. Contaminated acrylic resin samples brushed with antiseptic soap and rinsed with tap water had a mean of 0.32 colonies, whereas samples soaked for 20 seconds in 2% glutaraldehyde had a mean of 0.13 colonies.

The shellac samples contaminated with saliva, without further treatment, were found to have a mean of 240 colonies at a 10^{-4} dilution. The samples contaminated with saliva and rinsed with tap water had a mean of 6.32 colonies. The contaminated shellac samples brushed with antiseptic soap and rinsed with tap water had a mean of 0.41 colonies, while the samples soaked for 20 seconds in 2% glutaraldehyde had a mean of 0.14 colonies.

Ceramic samples contaminated with saliva and without further treatment were found to have a mean of 330 colonies at a 10^{-4} dilution. Ceramic samples contaminated with saliva and rinsed with tap water had a mean of 7 colonies. Contaminated ceramic samples brushed with antiseptic soap and rinsed with tap water had a mean of 0.32 colonies, whereas samples soaked for 20 seconds in 2% glutaraldehyde had a mean of 0.05.

The metal alloy samples contaminated with saliva and without further treatment had a mean of 329 colonies at 10^{-4} dilution. The metal alloy samples contaminated with saliva and rinsed with tap water had a mean of 9.68 colonies. The

Table 2. Count of Bacteria Colonies Observed After 24 Hours

Dental Materials	Treatment	Mean	Number	SD	Median	Minim	Maxim
Acrylic	Glutaraldehyde	0.1364	22	0.4676	0.0000	0.00	2.00
	Soap + Water	0.3182	22	0.7799	0.0000	0.00	3.00
	Water	5.4091	22	10.6398	0.5000	0.00	45.00
	Saliva	259.0909	22	131.5081	225.0	150.0	600.0
	Total	66.2386	88	129.4078	0.0000	0.0	660.00
Shellac	Glutaraldehyde	0.1364	22	0.4676	0.0000	0.00	2.00
	Soap + Water	0.4091	22	1.3331	0.0000	0.00	6.00
	Water	6.3182	22	13.3466	1.000	0.00	60.00
	Saliva	240.9091	22	146.9016	200.0	100.0	700.00
	Total	61.9432	88	126.7187	0.0000	0.00	700.00
Ceramic	Glutaraldehyde	4.545E-02	22	0.2132	0.0000	0.00	1.00
	Soap + Water	0.3182	22	0.6463	0.0000	0.00	2.00
	Water	7.0000	22	11.0151	3.0000	0.00	40.00
	Saliva	330.9524	22	135.5325	300.0	150.0	650.00
	Total	81.7471	88	155.8828	0.0000	0.00	650.00
Metal Alloy	Glutaraldehyde	9.091E-02	22	0.2942	0.0000	0.00	1.00
	Soap + Water	1.2273	22	2.3691	0.0000	0.00	10.00
	Water	9.6818	22	13.6011	2.000	0.00	45.00
	Saliva	329.5455	22	133.3266	300.0	100.0	600.00
	Total	85.1364	88	156.4975	1.000	0.00	600.00

Table 3. Mean Number of Bacterial Colonies Observed in Each Dental Material

Dental Materials	Contamination with Saliva, No Cleaning	Rinsed in Tap Water	Brushed with Antiseptic Soap	Immersed in Glutaraldehyde
Acrylic resin denture base	259	5.41	0.32	0.1
Shellac	240	6.32	0.41	0.1
Ceramic	330	7	0.32	0.0
Metal alloy	329	9.68	1.23	0.0

contaminated metal alloy samples brushed with antiseptic soap and rinsed with tap water had a mean of 1.23 colonies, while the samples soaked for 20 seconds in 2% glutaraldehyde had a mean of 0.09 colonies.

The number of colonies growing on the ceramic and metal alloy surfaces due to saliva contamination was statistically significant compared to that on the acrylic surfaces and shellac ($P < .05$).

The bacterial counts of the samples were significantly reduced by rinsing all treated dental materials with tap water for 20 seconds after contamination with saliva ($P < .05$). The ANOVA results showed a significant difference between all saliva-contaminated dental material samples disinfected with 2% glutaraldehyde or rinsed with antiseptic soap, compared to those rinsed only with water or those subjected to no cleaning treatments (Table 3).

DISCUSSION

Patient safety is a critical issue in healthcare delivery, and preventing contamination during treatment is just as vital as providing healthcare services. Given the potential for transmission of infectious diseases through blood and saliva, dentists are exposed to a wide variety of microorganisms, either directly or indirectly. The oral cavity serves as a constant reservoir of microorganisms, capable of facilitating cross-infections by transferring pathogens.[17,29]

Cross-infection control has become increasingly important in dentistry, particularly during the COVID-19 pandemic. The risk of cross-contamination is closely related to the communication and contact between dental staff and the dental laboratory. The use of a large number of materials and instruments during dental treatment, as well as direct contact with saliva and blood, further increases the risk.[16]

The transfer of microorganisms that may cause infection among patients, dentists, and dental clinic personnel is known as cross-infection. Prosthodontics, in particular, is one of the most common areas of cross-infection between dental laboratories and dental clinics. It is crucial to assess and improve compliance with standard infection control guidelines, especially in the prosthodontics clinic.[30]

Because the construction of prosthetic restorations requires multiple sessions, there is a high risk of cross-infection from dental materials that are constantly transported between dental clinics and laboratories.[16,22,31]

Prosthesis construction comprises various stages, including making impressions, obtaining a cast, applying occlusal wax rims, fitting metal and ceramic crowns, attempting removable total and partial prostheses, and fitting the finished prosthesis into the mouth. If these materials are not disinfected after their use at every appointment, they pose a risk of cross-infection between the dental clinic and the dental laboratory. Communication between the dentist and the dental laboratory regarding the disinfection of these materials is also extremely important to prevent repeated disinfection and to protect the surfaces of the materials.[4,32]

In a previous study, opportunistic bacteria were found in 67% of denture materials sent from dentists' offices to the dental laboratory.[20]

Dental impressions, prostheses, and other dental materials are a potential source of contamination as they come into contact with patients' oral secretions (blood, saliva), which can harbor a large number of microorganisms.[17,29]

Other research has confirmed that dental laboratory workers and dentists should implement adequate infection control procedures for all dental materials sent to or received from dental clinics by laboratories.[21]

Another study confirmed that 60% of dental materials transferred from a dental laboratory to a clinic contained microorganisms.[33]One study reported that 9 out of 10 sterile complete dentures sent to various dental laboratories were contaminated with pathogenic microorganisms.[34]

Therefore, it is essential to disinfect orthodontic appliances, prosthetic appliances, implant parts, impression materials, impression trays, occlusal wax, and denture base materials that may cause cross-infection between dental clinics and laboratories.[35,36]

Dental impressions taken from patients should be disinfected to prevent cross-contamination of dental casts. Research has shown that rinsing dental impressions under running water can remove 40%-90% of bacteria from the surface.[37]

However, dental casts may become reinfected when acrylic resin denture bases, metal alloys, or ceramics are placed in the patient's mouth and then reinserted into the dental casts.

In this study, the methods of disinfection used are rinsing with tap water, scrubbing with a soft toothbrush for 20 seconds using antiseptic hand soap, and immersion in 2% glutaraldehyde solution after saliva contamination. In this study, undiluted saliva was utilized due to its propensity to serve as a surrogate for the actual contaminants that would be encountered with the dental materials and dental casts used in a clinical setting.

In this study, bacterial growth persisted for at least 24 hours on dental materials, including acrylic resin denture bases, shellac, metal, and ceramic, contaminated with saliva. Additionally, it was observed that rinsing all dental materials with cold tap water for 20 seconds significantly reduced the number of bacterial colonies compared with dental materials that were not cleaned after contamination ($P < .05$).

A previous study showed that rinsing dental casts under running tap water for 20 seconds decreased bacterial growth.[19]Another study reported surface damage to dental casts rinsed with tap water for 15 minutes.[38]However, disinfecting the dental cast at every stage of prosthesis construction may deform it.[39,40] In this study, water was used as a disinfectant because it is readily available at the point of care and easily accessible to all practitioners. However, the utilization of tap water alone proved to be ineffective in significantly reducing the colonies, as evidenced by the findings of this study.

The present study showed that disinfecting dental materials before placing them in a cast is more effective than disinfecting the cast itself multiple times. Even rinsing with tap water for 20 seconds significantly reduced the number of bacterial colonies. In this study, a 20-second process was used for all steps to provide adequate disinfection in the shortest time possible. In a previous study, dental casts were disinfected by rinsing with tap water, followed by soap and running water, and then soaking them in a 2% glutaraldehyde solution for 20 seconds.[19]Another study evaluated bacterial growth after holding dental casts in different disinfectants for 5 minutes.[41]

In both studies, only the dental cast was cleaned and disinfected. In this study, the aim was to avoid multiple disinfections of the same cast by applying cleaning and disinfection procedures to 4 different dental prosthesis materials.

Various agents have been proposed to disinfect dentures. An ideal disinfectant should be inexpensive, effective against harmful microorganisms, non-toxic, and should not damage denture surfaces.[42,43]

Dentists and dental technicians should be able to easily and quickly implement disinfection processes. The results of the present study support this finding. Almost no bacterial colonies remained after the samples were rinsed with antiseptic soap or soaked in 2% glutaraldehyde solution.

The number of colonies growing on ceramic and metal alloy surfaces due to saliva contamination was found to be statistically significant when compared to acrylic samples and shellac ($P < .05$) (Table 3).

Microbial adhesion on acrylic surfaces is closely related to the physical properties of the material. Acrylic resins can provide a suitable environment for microbial colonization due to their porous structure. However, the presence of saliva has been shown to reduce the adhesion of C. albicans, thereby mitigating the impact of surface roughness.[44,45]Another study reports that the rough surface showed a greater tendency for plaque accumulation than the smooth surface.[46,47]

The ceramic samples used in this study were not glazed. Glazing is not applied to ceramics in the dentin try-in process. In a previous study, a positive correlation was observed between surface roughness and plaque accumulation. This finding aligns with the results of this study.[48]

This important finding once again underscores the importance of disinfection, given the risk of increased bacterial adhesion during dentin try-in in clinical practice.

The metal alloy samples were leveled and polished to a smooth finish. Many factors influence bacterial attachment, accumulation, and plaque formation in dental casting alloys. In vitro investigations have demonstrated that alloys comprising copper and silver, both of which are released, exhibit enhanced antimicrobial activity in comparison to specific base metal alloys.[49]

In a study by Akça et al[50] investigating bacterial attachment in different fixed prosthetic restoration materials, it was reported that among the materials used under surface porcelains (Cr-Ni, Empress 2 ingot and InCeram), Cr-Ni alloy allowed the least bacterial attachment.[51]

However, the high number of bacteria may be due to invisible micropores in the metal samples, and further research on this topic is required. Additionally, it is necessary to investigate whether any damage occurs on the surfaces of these dental materials due to brushing with soap and rinsing water, or soaking in 2% glutaraldehyde for 20 seconds.

Upon evaluation of the results, it is observed that saliva is retained on the surface of dental materials, leading to bacterial growth over time (Table 3). The dental materials used to make dentures can cause cross-infections between dental clinics and laboratories. To prevent this, it is important to disinfect the dental materials during denture fabrication. At a minimum, rinsing these materials with running tap water after trying them in the patient's mouth can reduce bacterial growth; however, this method cannot be considered an adequate or reliable method for disinfection, as it may sometimes

lead to further contamination due to contaminants in the tap water. The most efficacious cleaning and disinfection method was determined to be brushing with soap and rinsing with tap water and immersion in 2% glutaraldehyde for 20 seconds.

CONCLUSION

Within the limitations of this current study, it was concluded that:

1. Even simply rinsing the 4 different dental materials used in this study under running water for 20 seconds after removing them from the patient's mouth significantly reduced the bacterial growth.
2. Brushing these materials with antiseptic soap under running water reduced the bacterial count more effectively.
3. Almost no bacterial growth was observed when these materials were immersed in a 2% glutaraldehyde solution for 20 seconds.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Artificial Intelligence Usage Statement: The authors declared that no Artificial Intelligence tool was used in the preparation of the manuscript.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of University of Health Science Ethics Commitee of Ankara Yıldırım Beyazıt University (Approval No.: 09/982; Date: November 21, 2024).

Informed Consent: Verbal/Written informed consent was obtained from the patients/patient who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Acknowledgment: The author acknowledges and thanks Microbiologist Dr. Hatice Akar for her contribution to the microbiological tests and statistical analysis of the data.

Declaration of Interests: The author has no conflict of interest to declare.

Funding: The author declared that this study has received no financial support.

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