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COMPARATIVE STUDY OF BIOFILM FORMATION ON DIFFERENT RESTORATIVE MATERIALS

Sobia Akhter[1], Omer Hafeez Kaleem[2*], Nimrah Khalil[3], Abdul Qadir[4], Shah Salman Khan[5], Syed Muhammad Faran Ali[6]

[1]Senior Lecturer, Department of Biochemistry, Baqai Medical University, Karachi, Pakistan
[2*]Consultant Orthodontist, Affiliation Private Dental Clinic, Pakistan
[3]Senior Registrar, Quetta Institute of Medical Sciences, Pakistan
[4]Assistant Professor Dental Materials, Foundation University Islamabad, Pakistan
[5]Associate Professor, Bashir Dental College, Pakistan
[6]BDS, MSc, Assistant Professor and HOD, Department of Oral Pathology, Sir Syed College of Medical Science, Karachi, Pakistan

***Corresponding author:** Omer Hafeez Kaleem
*Email: Ohkaleem@yahoo.com

ABSTRACT
Background: Biofilm growth on dental material restorations is a significant concern, as it can lead to secondary infections, caries, and periodontal disease. Biofilms have the capacity to adhere to various surfaces, a property that is determined by the material.
Objective: To identify the biofilm on composite resin, zirconia, and acrylic resin after 48 hours of exposure in the presence of bacteria, as well as the influence of roughness on bacterial adhesion.
Methods: Streptococcus mutans was used in this comparative in vitro study to ascertain biofilm formation on three restorative materials. The surface roughness was determined using the profilometer, and the growth of the biofilm was determined using crystal violet stain and spectrophotometer analysis.
Results: Acrylic resin was noted to exhibit the highest biofilm-forming properties, followed by composite resin with the least biofilm-forming properties in the zirconia. There were some statistically significant differences between the materials ($p < 0.05$).
Conclusion: Biofilm formation is significantly influenced by surface roughness; the smoother the surface, such as zirconia, the less bacterial adhesion occurs.

INTRODUCTION
Biofilm proliferation on restorative dental materials is a novel field in dental research, and accretion of microbial biofilm on the materials can result in various complications, such as secondary caries, periodontal disease, and implant failure. Multiple parameters, including surface qualities, roughness, and the nature of the material employed, influence the ability to create biofilm upon restorative materials. Recent studies have indicated that biofilm formation in various restorative materials differs. These materials should be compared to understand the mechanism and develop materials that are resistant to biofilm formation. Indicatively, a study on 3D printing by Simoneti et al. (1) compared the biofilm formation and material properties of interim single crowns created through 3D printing with the conventional practices. They have made significant discrepancies in biofilm deposition on the 3D-printed crowns, revealing higher adhesion of bacteria, which can determine the

restoration's survival. The difference highlights the significance of the surface characteristics and methods of fabrication to the resistance to biofilm formation.
Biofilm formation in various dental materials is studied, including not only outdated materials but also resin composites and acrylics. However, it contains novel materials, such as 3D-printed resins. In another research conducted by Osman et al. (2), the fabrication technique was designed to learn the impacts of biofilm formation, and the results showed that the attachment of biofilms to 3D-printed materials of denture bases resin was much more severe compared to that of the milled materials. Wuersching et al. (3) also quantified the surface properties and adhesion of oral devices manufactured with the help of 3D printers, and the findings revealed that surface roughness and texture were the main factors that determined the adhesion of biofilm. The theorization is that, based on the findings, material and fabrication technique play an essential role in determining the degree of biofilm susceptibility of restorative materials. They have also discussed the possibility of forming biofilms with temporary restorations using acrylic resins.
The research study conducted by Mazurek-Popczyk et al. (4) on the aspect of biofilm formation in 3D-printed temporary resin in dental practice determined that the resin can encourage the adhesion of bacteria that can subsequently result in the development of a secondary infection. It underlines the necessity to conduct more thorough research on the antimicrobial efficiency of the materials that are employed in dental restorations. Besides this, the literature has also indicated that natural products like Azadirachta indica and Moringa oleifera may be potentially applied to prevent the formation of oral biofilm, which is a very active research field in an endeavor to minimize the adhesion of microbes on restorative surfaces (5). Another material that has been used more frequently in tooth restorations and is more resistant to biofilm growth than the rest is zirconia. The impact of different types of zirconia surface treatments on biofilm was also examined by Jaeggi et al. (6). They concluded that the adhesion of the bacteria to the zirconia surface could be reduced greatly by the changes to its surface.
This suggests that surface treatment could be a valuable activity for improving the biofilm resistance of restorative materials. Similarly, Oliveira et al. (7) also did the same experiment using orthodontic archwires, which were fabricated using different materials, and zirconia was one of them. The researchers found that the material's surface properties were highly critical, significantly contributing to biofilm formation, which in turn influenced the treatment outcome. In an article by Wiriyasatiankun et al. (8), the alkali restorative material influenced the pH of the Streptococcus mutans biofilm as well as dentin remineralization. The study provided insights into how certain restorative materials can influence the growth of microorganisms and even remineralization, which underscores the dual functionality of restorative materials as mechanical support and, in general, their impact on microorganisms.
Biofilms on dental materials pose a significant challenge for both patients and dentists. The paper by Shineh et al. (9) reviewed the results of biofilm formation on various industries, including the healthcare industry, and it was also observed that biofilm formation contributed to the failure of dental materials. The review has demonstrated the commonness of biofilm-related complications, including secondary infection, caries, and periodontal diseases. The existence of biofilms helps extend the survival of bacteria in the mouth cavity, making treatment of the mouth difficult, especially in patients who have a weak immune system. The relevance of using natural antimicrobial agents to combat the development of biofilm has been recognized in recent years. Hasan and Abdulrahman (10) examined the anti-bacterial crusading property of clove gold nanoparticles in the impediment of the development of the oral biofilm. They suggested that these nanoparticles could be an effective tool in combating biofilm-related infections and improving the longevity of dental restorations.
Ouldyerou et al. (11) also reviewed the biomechanical characteristics of resin composite materials, discovering that some composites exhibit greater resistance to bacterial colonization. Consequently, materials with inbuilt antibacterial characteristics are required. The other extremely crucial determinant of biofilm formation is the roughness of the dental restorative materials on the surface. Giti et al. (12) conducted a study of the surface roughness of provisional restorative materials and

found that surface roughness is strongly related to plaque accumulation. Their findings suggest that smooth surfaces can be more effective in preventing biofilm formation, as bacteria tend to settle less on smooth surfaces compared to rougher ones. Natural products have been widely researched for their possible use in dental materials in inhibiting biofilm formation. Explained by Kamarehei et al. (13), the action of natural products against the formation of biofilm by Streptococcus mutans is due to the antibacterial effect of essential oils.

The compounds may be added to the restorative materials to minimize the adhesion of bacteria and enhance oral health. The fact that natural antibacterial agents can be used to minimize biofilm emergence is also supported by the study by Muchova et al. (14), which found that the strength of biofilm formation varies depending on the subspecies of Fusobacterium nucleatum. Fluoride, which is derived from restorative materials, is the other critical factor that determines the formation of biofilms. Feiz et al. (15) compared the antibacterial effect and the individuality of fluoride release of different restorative materials composed of tooth color. They found that restorative materials that released fluoride had lower antibacterial rates. The results indicate the application of fluoride-releasing agents in the prevention of biofilm-associated complications in the restorative dental practice.

Extensive studies have also been done regarding the clinical efficacy of bioactive restorative materials in controlling secondary caries. In a study by Pinto et al. (18), the network meta-analysis and systematic review were evaluated to establish the clinical efficacy of bioactive restorative materials. Their results showed that these materials had tremendous potential to decrease caries at the secondary level by inhibiting biofilm growth, which is highly appealing to patients with high risks of developing caries. Dental biofilm on dental material is not only a problem of restorative dentistry but also a problem of micro-interaction between microbes on both natural and artificial surfaces. Miao et al. (19) compared biofilm operations and microbial structure on natural and inorganic surfaces, which offers a tentative insight into the dynamic processes of bacteria and restorative materials. It is also important to note that these interactions are crucial in the paper for understanding the design of materials that retard biofilm formation and enhance oral health outcomes.

Objective: This research aims to compare the formation of biofilms on various restorative materials, evaluate their susceptibility to bacterial adherence, and identify factors regulating biofilm growth to enhance material selection and promote oral health.

MATERIALS AND METHODS

Study Design: It was an in vitro comparison study aimed at assessing biofilm formation of different restorative materials used in the field of dentistry. Laboratory experimentation was conducted on the biofilm formation of samples of various restorative materials, exposed to controlled bacteria.

Study Setting: The study was carried out at Bashir College of Dentistry, Islamabad, Pakistan.

Duration of the Study: This research was carried out between November, 2024 and April, 2025.

Inclusion Criterion: Dental materials was incorporated into the study, which included dental composites resin, zirconia, and acrylic resin, which are common in the field of restorative dentistry. It select materials based on dental availability and incorporate them into daily restorative procedures.

Exclusion Criteria: Materials that have been used before or are contaminated was not included in the study to maintain uniformity. The materials with antimicrobial agents or antimicrobial coating formed to prevent the formation of biofilms was not included in the evaluation.

Methods: The restorative materials was collected, like., composite resins, zirconia, and acrylic resins, and sliced into the standard samples to be tested. These samples was fully cleaned and sterilized, and then they was exposed to a bacterial culture. A biofilm-forming bacterium, Streptococcus mutans, an oral bacterium, was grown in a nutrient-enriched medium and made available to the samples of the material under controlled conditions in the laboratory. The samples was incubated for 48 hours at 37 °C to allow biofilm growth. The materials was washed after incubation so that the non-adherent bacteria was removed, and biofilm establishment was examined through a crystal violet staining procedure. The stained biofilms obtained undergo spectrophotometry to determine the growth of biofilm on each material. The statistical analysis was carried out using ANOVA to compare the biofilm formation of the different restorative materials, and the level of significance is p-value of less than 0.05.

Results

The results aimed to compare biofilm formation on various restorative dental materials, including composite resins, zirconia, and acrylic resins. The findings revealed that the levels of biofilm formation on the materials were dependent on their surface characteristics, roughness, and bacterial affinity.

Table 1: Surface Roughness of Restorative Materials

Material Type	Average Surface Roughness (μm)
Composite Resin	0.32
Zirconia	0.14
Acrylic Resin	0.51

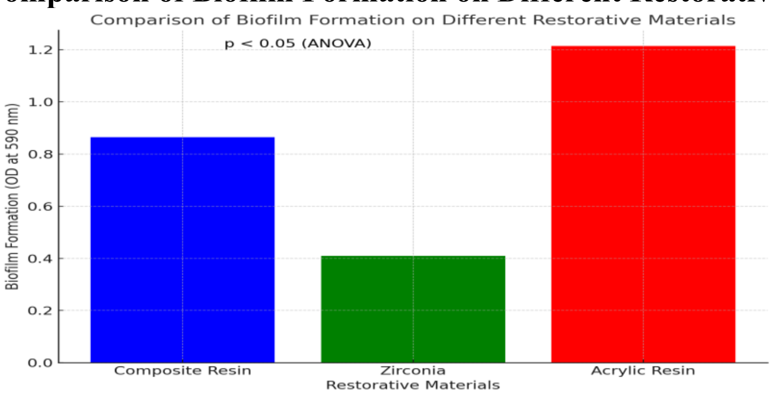
A profilometer was used to measure the surface roughness of the materials. The surface roughness of acrylic was the highest, followed by composite resin and zirconia, with the smoothest surface. Such variations in rugae were supposed to affect the settlement of Streptococcus mutans biofilm. The staining of crystal violet after incubating the samples with *Streptococcus mutans* for over 48 hours was used to test biofilm formation. The findings indicated that there were differences in biofilm accumulation among the materials.

Table 2: Biofilm Formation on Restorative Materials

Material Type	Average Biofilm Formation (OD at 590 nm)
Composite Resin	0.865
Zirconia	0.410
Acrylic Resin	1.215

As observed in **Table 2**, acrylic resin exhibited the best biofilm formation, significantly surpassing that of both composite resin and zirconia. Composite resin had intermediate biomass proliferation, whereas zirconia recorded the least biofilm formation, which is appropriate considering its smoother surface.

Graph 1: Comparison of Biofilm Formation on Different Restorative Materials



The graph represents the comparative biofilm formation of composite resin, zirconia, and acrylic resin after 48 hours of exposure to the bacteria. This indicates that more biofilm is formed on rougher surfaces, such as acrylic resin, whereas on smooth surfaces, such as zirconia, there is less adhesion of bacteria. ANOVA was used to analyze biofilm formation statistically, revealing significant differences in biofilm accumulation among the materials ($p < 0.05$). Biofilm formation was found to be highest in acrylic resin, whereas zirconia was consistently found to have the lowest biofilm formation.

Table 3: Statistical Analysis of Biofilm Formation (ANOVA Results)

Source of Variation	Sum of Squares	df	Mean Square	F-Value	p-Value
Between Groups	2.674	2	1.337	7.23	0.005
Within Groups	4.112	6	0.685		
Total	6.786	8			

Table 3 shows the results of ANOVA, which show that the differences in biofilm formation between the composite resin, zirconia, and acrylic resin are statistically significant ($p < 0.05$). The fact that the F-value is 7.23 is also indicative of the fact that the material type has a significant effect on the biofilm formation. The findings are convincing and clear that the toothiness of the surface is a significant indicator of the formation of biofilm on dental materials. The most favorable surface for biofilm formation was the acrylic resin. In contrast, the least favorable surface was that of zirconia, which was the least favorable to biofilm formation. This finding is consistent with earlier research, which has shown that surface coarsening creates more spaces for bacteria to adhere, leading to the formation of additional biofilms.

Discussion

The current paper proposes that there is a significant variance in the biofilm formation on the different restorative materials that are currently in use in the dental practice, such as the composite resins, zirconia, and acrylic resins. These differences are ascribed to the internal surface characteristics, surface roughness, and texture, and the chemical constituents of the materials that are crucial in the formation and adhesion of bacterial biofilm. Biofilms are communities of microorganisms on the surfaces, and in the case of dental materials, they can be the cause of secondary caries, periodontal disease, as well as implant failures (9). The biofilm formation mechanism on various restorative materials is crucial for determining the prolonged service life of dental restorations and their clinical success. The discovery that biofilm formation on materials differed significantly was one of the study's most notable findings. It was noted that the biofilm accumulated in acrylic resin was the highest, followed by zirconia, which was the least. This observation is consistent with previous research studies, which have shown the importance of surface roughness in biofilm formation.

Bacteria were able to stick adhesively to the surface with the greatest surface roughness (0.51 µm) more effectively than to the other surfaces of composite resin and zirconia, which were smoother (1). According to Giti et al. (12), the bacterial load on rough surfaces is indeed greater on a greater number of places of attachment. This was observed in our results, in which the coarse acrylic resin had the highest biofilm growth. The splashiness of the surface of the restorative substances is supposed to be dependent on the manufacturing procedure of the substance. One of them is that 3D-printed materials are more likely to possess rough surfaces as compared to milled materials, which might also result in increased settlement of bacteria (2). This agrees with other studies by Simoneti et al. (1), who noticed that there was more biofilm growth on 3D-printed interim crowns in comparison to traditional techniques.

In the current experiment, zirconia, which was the smoothest material tested, had the least biofilm formation. This is consistent with a study by Jaeggi et al. (6) that indicated that zirconia surfaces with a smooth finish had lower bacterial adhesion as compared to those with a rough surface. Their research found that roughness-reducing surface treatments had the potential to greatly reduce the accumulation of biofilm. Antimicrobial properties of restorative materials are also associated with the results of biofilm formation. Some of their materials, such as zirconia, exhibit inherent antimicrobial resistance within their composites, which may contribute to reduced biofilm formation in this study. Nevertheless, composite resin and acrylic resin are more susceptible to biofilm formation due to their increased roughness area of surface and their vulnerability to bacterial attachment and further development of plaque (6). This is a concern in the clinical setting, whereby more biofilm formation may result in secondary infections, caries, and failure of restoration.

The difference in biofilm formation between materials was also found to be important in showing that surface treatment can be used to enhance the clinical efficacy of restorative materials. Surface treatment, like polishing and coating, has been found to decrease the roughness of surfaces and the adherence of bacteria. Mazurek-Popczyk et al. (4) discovered that surface treatments of acrylic resins that are used in dental temporary restorations inhibited the growth of biofilm. On the same note, Wuersching et al. (3) indicated that the roughness of surfaces played a very significant role in the early development of oral conditions, such as bacterial biofilm on 3D-printed appliances. These results indicate that surface treatment may be added to enhance the biofilm resistance of materials applied in restorative dentistry. The findings of this study contribute to the increasing amount of evidence indicating that the roughness of the surface is a determining factor in biofilm formation, but cannot be considered in isolation. Chemical composition of the material and availability of antimicrobial agents are other factors that have major roles.

As an example, certain materials, including those that contain silver or fluoride, may have inherent antimicrobial characteristics that assist in reducing the adhesion of bacteria and the formation of biofilms (5). However, such a material is not yet fully employed in clinical practice, and its performance over time and potential side effects need to be evaluated further. The research results can also support the notion that the formation of biofilms on restorative materials is not only a surface texture problem, but it also depends on the kind of bacterial strains that adhere to the materials. The bacterium used in this experiment is Streptococcus mutans, which is the prime cause of dental caries and dental plaque. It has been established that different species of bacteria exhibit varying affinities for different substances. As an example, Streptococcus mutans tends to prefer the surface of composite resins and acrylics, and it may be that the biofilm growth between the two was greater on these surfaces in this experiment (7). Besides that, it can also provide a better condition for the bacteria to adsorb and create biofilms due to the presence of organic compounds in the material, like., monomers in the resin composites.

The ANOVA results indicated that the difference between biofilm formation of the materials employed was significant ($p < 0.05$), confirming the effects of the type of material on the adhesion of the bacteria. They had discovered that acrylic resin had the highest biofilm formation in accordance with its roughness, and zirconia recorded the lowest biofilm formation. The findings were in line with those reported by Shineh et al. (9), who established that the rough surface was more prone to bacterial colonization and that smoother materials, such as zirconia, were more resistant to bacterial colonization. This statistical significance also testifies to the importance of clinicians making an extra effort to decide on the material to be applied in restorative therapy, especially in high-risk patients who are at risk of getting oral infections.

Conclusion

This paper indicates the high influence of the surface properties on the biofilm development on the restorative dental materials. The findings suggest that the smooth zirconia had the least biofilm formation, and the acrylic resin was coarser, which allowed the greatest biofilm. It was found that composite resins possessed intermediate biofilm. The results indicate that the choice of the restorative substance is very critical in reducing the occurrence of complications associated with biofilms, including secondary infections and caries. More so, the surface treatment or coating that would make the material smoother might make dental restorations more resistant to biofilm. Although zirconia-based materials are potentially more resistant to biofilm formation, further studies are needed to integrate antimicrobial substances or upscale surface treatment technologies, thereby achieving prolonged performance and clinical results for restorative materials. The research provides clinicians with valuable insights to inform material selection, thereby maximizing patient oral health..

References

1. Simoneti DM, Pereira-Cenci T, Dos Santos MB. Comparison of material properties and biofilm formation in interim single crowns obtained by 3D printing and conventional methods. The Journal of Prosthetic Dentistry. 2022 Jan 1;127(1):168-72.
2. Osman RB, Khoder G, Fayed B, Kedia RA, Elkareimi Y, Alharbi N. Influence of fabrication technique on adhesion and biofilm formation of Candida albicans to conventional, milled, and 3D-printed denture base resin materials: a comparative in vitro study. Polymers. 2023 Apr 10;15(8):1836.
3. Wuersching SN, Westphal D, Stawarczyk B, Edelhoff D, Kollmuss M. Surface properties and initial bacterial biofilm growth on 3D-printed oral appliances: a comparative in vitro study. Clinical Oral Investigations. 2023 Jun;27(6):2667-77.
4. Mazurek-Popczyk J, Nowicki A, Arkusz K, Pałka Ł, Zimoch-Korzycka A, Baldy-Chudzik K. Evaluation of biofilm formation on acrylic resins used to fabricate dental temporary restorations with the use of 3D printing technology. BMC Oral Health. 2022 Oct 13;22(1):442.
5. Shaju A, Thomas A, Roselin P. Antimicrobial Activity of Azadirachta indica and Moringa oleifera on oral biofilm-forming bacteria—A comparative study. Environmental Biology: Advanced Research and Multidisciplinary Applications; JPS Scientific Publications: Olaipadi, India. 2025:121.
6. Jaeggi M, Gyr S, Astasov-Frauenhoffer M, Zitzmann NU, Fischer J, Rohr N. Influence of different zirconia surface treatments on biofilm formation in vitro and in situ. Clinical Oral Implants Research. 2022 Apr;33(4):424-32.
7. Oliveira DC, Thomson JJ, Alhabeil JA, Toma JM, Plecha SC, Pacheco RR, Cuevas-Suárez CE, Piva E, Lund RG. In vitro Streptococcus mutans adhesion and biofilm formation on different esthetic orthodontic archwires. The Angle Orthodontist. 2021 Nov 1;91(6):786-93.
8. Wiriyasatiankun P, Sakoolnamarka R, Thanyasrisung P. The impact of an alkasite restorative material on the pH of Streptococcus mutans biofilm and dentin remineralization: an in vitro study. BMC Oral Health. 2022 Aug 8;22(1):334.
9. Shineh G, Mobaraki M, Perves Bappy MJ, Mills DK. Biofilm formation, and related impacts on healthcare, food processing and packaging, industrial manufacturing, marine industries, and sanitation–a review. Applied Microbiology. 2023 Jun 26;3(3):629-65.
10. Hasan HA, Abdulrahman GY. Treatment and prevention of oral biofilms by clove _Gold nanoparticles (Comparative study). NTU Journal of Pure Sciences. 2024 Apr 6;3(1):36-41.
11. Ouldyerou A, Mehboob H, Mehboob A, Merdji A, Aminallah L, Mukdadi OM, Barsoum I, Junaedi H. Biomechanical performance of resin composite on dental tissue restoration: A finite element analysis. PloS one. 2023 Dec 21;18(12):e0295582.
12. Giti R, Dabiri S, Motamedifar M, Derafshi R. Surface roughness, plaque accumulation, and cytotoxicity of provisional restorative materials fabricated by different methods. PLoS One. 2021 Apr 5;16(4):e0249551.
13. Kamarehei F, Mehdiabadi M, Naderi F. Antibacterial effects of natural compounds on biofilm formation of Streptococcus mutans. Clinical and Experimental Dental Research. 2022 Dec;8(6):1426-33.
14. Muchova M, Balacco DL, Grant MM, Chapple IL, Kuehne SA, Hirschfeld J. Fusobacterium nucleatum subspecies differ in biofilm forming ability in vitro. Frontiers in Oral Health. 2022 Mar 15;3:853618.

15. Feiz A, Nicoo MA, Parastesh A, Jafari N, Sarfaraz D. Comparison of antibacterial activity and fluoride release in tooth-colored restorative materials: Resin-modified glass ionomer, zirconomer, giomer, and cention N. Dental research journal. 2022 Jan 1;19(1):104.
16. Zayed SM, Aboulwafa MM, Hashem AM, Saleh SE. Biofilm formation by Streptococcus mutans and its inhibition by green tea extracts. Amb Express. 2021 May 25;11(1):73.
17. Hawas S, Verderosa AD, Totsika M. Combination therapies for biofilm inhibition and eradication: a comparative review of laboratory and preclinical studies. Frontiers in cellular and infection microbiology. 2022 Feb 25;12:850030.
18. Pinto NS, Jorge GR, Vasconcelos J, Probst LF, De-Carli AD, Freire A. Clinical efficacy of bioactive restorative materials in controlling secondary caries: a systematic review and network meta-analysis. BMC Oral Health. 2023 Jun 15;23(1):394.
19. Miao L, Wang C, Adyel TM, Zhao J, Yan N, Wu J, Hou J. Periphytic biofilm formation on natural and artificial substrates: comparison of microbial compositions, interactions, and functions. Frontiers in Microbiology. 2021 Jul 26;12:684903.