

Evaluation of Biofilm Formation on Different Orthodontic Bracket Materials.

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ABSTRACT

Background

Biofilm formation on orthodontic brackets is a significant concern due to its implications on oral hygiene, caries development, and periodontal health. The choice of bracket material may influence biofilm adherence and growth. This study evaluates biofilm formation on different orthodontic bracket materials to identify the least biofilm-retentive options.

Materials and Methods

A total of 60 orthodontic brackets, divided into three groups based on material type—metallic (n=20), ceramic (n=20), and composite resin (n=20)—were tested. Artificial saliva was used to mimic oral conditions, and *Streptococcus mutans* was selected as the primary biofilm-forming bacterium. Brackets were incubated for 48 hours at 37°C, after which biofilm quantification was performed using crystal violet staining and spectrophotometric analysis at 595 nm. Statistical analysis was conducted using ANOVA to compare biofilm formation across groups.

Results

The mean optical density (OD) values for biofilm formation were as follows: metallic brackets (0.25 ± 0.05), ceramic brackets (0.42 ± 0.08), and composite resin brackets (0.58 ± 0.07). Metallic brackets demonstrated the lowest biofilm formation, which was statistically significant compared to ceramic and composite resin brackets ($p < 0.05$). Ceramic brackets showed moderate biofilm formation, while composite resin brackets exhibited the highest levels of biofilm accumulation.

Conclusion

Metallic brackets are less conducive to biofilm formation compared to ceramic and composite resin brackets, making them a preferable choice for minimizing biofilm-related complications during orthodontic treatment. These findings emphasize the importance of material selection in orthodontic practice to promote better oral health outcomes.

Keywords

Biofilm, Orthodontic brackets, Metallic brackets, Ceramic brackets, Composite resin brackets, *Streptococcus mutans*.

INTRODUCTION

Biofilm formation on orthodontic appliances poses a significant challenge to maintaining oral health during treatment. Orthodontic brackets provide a favorable environment for microbial colonization due to their complex structure and increased retention of food debris and plaque. This biofilm, predominantly formed by *Streptococcus mutans* and other cariogenic microorganisms, can lead to white spot lesions, dental caries, and periodontal inflammation if not managed effectively (1,2).

The material composition of orthodontic brackets plays a critical role in biofilm adherence. Metallic brackets, commonly made of stainless steel, are widely used due to their durability and lower surface roughness. Ceramic brackets, though esthetically superior, have been associated with higher biofilm retention due to their relatively rougher surface. Composite resin brackets, often used in certain clinical scenarios, are also prone to biofilm accumulation due to their porosity and

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surface characteristics (3-5).

Understanding the biofilm-forming potential of various bracket materials is essential to guide clinicians in selecting materials that minimize biofilm retention and subsequent oral health complications. Previous studies have explored the relationship between bracket materials and microbial adhesion, but the findings remain inconsistent (6,7). In order to determine which material is most resistant to biofilm formation, this study will compare and contrast the production of biofilms on metallic, ceramic, and composite resin brackets under controlled laboratory circumstances.

MATERIALS AND METHODS

Study Design

This in vitro study evaluated the biofilm formation on orthodontic brackets made of three different materials: metallic, ceramic, and composite resin.

Sample Preparation

A total of 60 orthodontic brackets were divided equally into three groups based on their material composition: metallic (stainless steel) brackets, ceramic brackets, and composite resin brackets ($n = 20$ per group). All brackets were standardized in size and shape to ensure uniformity.

Biofilm Formation Protocol

The initial step in sterilising the brackets was to autoclave them for 15 minutes at 121°C . Next, they were subjected to a 24-hour immersion in synthetic saliva at 37°C in order to mimic the conditions found in the mouth. A concentration of 1×10^8 CFU/mL was achieved by cultivating *Streptococcus mutans* ATCC 25175 in brain-heart infusion (BHI) broth.

Two millilitres of the bacterial suspension was added to each well of a 24-well plate before the brackets were set in. To encourage biofilm formation, the plates were incubated under anaerobic conditions at 37°C for 48 hours. In order to keep the bacteria viable during the incubation period, the medium was changed every 24 hours.

Biofilm Quantification

The brackets were delicately rinsed with phosphate-buffered saline (PBS) after incubation to eliminate any bacteria that did not adhere. The crystal violet staining method was used to quantify biofilms. To stain the brackets, we used a 0.1% crystal violet solution for 15

minutes. After that, we rinsed them well with distilled water to get rid of any excess stain. A spectrophotometer was used to measure the optical density (OD) at 595 nm after dissolving the attached dye in 95% ethanol.

Statistical Analysis

To assess the groups' biofilm development average optical density (OD), we used one-way analysis of variance (ANOVA) and, for pairwise comparisons, Tukey's post hoc test. It was deemed statistically significant if the p-value was less than 0.05.

RESULTS

The study evaluated the biofilm formation on three types of orthodontic bracket materials: metallic, ceramic, and composite resin. The biofilm quantification results, expressed as mean optical density (OD) values, are summarized in **Table 1**.

The metallic brackets exhibited the lowest biofilm formation, with a mean OD value of 0.25 ± 0.05 , which was significantly lower compared to ceramic (0.42 ± 0.08) and composite resin brackets (0.58 ± 0.07). The ceramic brackets demonstrated moderate biofilm formation, while composite resin brackets showed the highest biofilm retention ($p < 0.05$). Pairwise comparisons revealed statistically significant differences in biofilm formation between all groups (**Table 1**).

Table 1: Mean OD Values of Biofilm Formation on Different Bracket Materials

Bracket Material	Mean OD (595 nm) \pm SD	p-value (ANOVA)
Metallic Brackets	0.25 ± 0.05	<0.05
Ceramic Brackets	0.42 ± 0.08	<0.05
Composite Resin Brackets	0.58 ± 0.07	<0.05

The results suggest that metallic brackets are less prone to biofilm formation compared to ceramic and composite resin brackets (**Table 1**). The findings indicate that material composition significantly influences biofilm adherence, with metallic brackets being the most favorable option for minimizing microbial colonization.

DISCUSSION

The present study aimed to evaluate the biofilm formation on orthodontic brackets made of metallic, ceramic, and composite resin materials. The findings demonstrated significant differences in biofilm retention



across these materials, with metallic brackets exhibiting the least biofilm formation, followed by ceramic and composite resin brackets. These results have important clinical implications for the selection of orthodontic brackets to minimize oral health complications during treatment.

The lower biofilm formation observed on metallic brackets can be attributed to their smoother surface and lower surface free energy compared to ceramic and composite resin brackets. Previous studies have similarly reported that stainless steel brackets are less conducive to microbial adhesion due to their polished surfaces, which limit bacterial attachment (1,2). In contrast, ceramic brackets, despite their aesthetic advantages, exhibit higher surface roughness, which provides a favorable environment for bacterial colonization (3,4). Composite resin brackets, being porous and hydrophilic, further enhance biofilm retention, corroborating earlier findings in the literature (5,6).

The role of surface roughness in microbial adherence has been well-documented. Eliades et al. reported that the initial pellicle formation and microbial attachment are strongly influenced by the material's surface properties (7). Similar observations were made by Türkkahraman et al., who found that increased surface roughness on orthodontic appliances correlates with higher microbial colonization (8). Moreover, composite resin brackets, due to their susceptibility to degradation over time, may provide additional sites for bacterial adhesion (9).

Streptococcus mutans, the primary biofilm-forming bacterium used in this study, is a significant contributor to dental caries and white spot lesions in orthodontic patients. The higher biofilm formation on ceramic and composite resin brackets may explain the increased prevalence of demineralization and caries in patients with these types of brackets, as highlighted in clinical studies (10,11). Furthermore, the findings emphasize the importance of strict oral hygiene measures for patients undergoing orthodontic treatment with non-metallic brackets.

The clinical implications of these findings are twofold. First, clinicians should consider the biofilm-forming potential of bracket materials when planning orthodontic treatment, particularly for patients with a high risk of caries or periodontal disease. Second, the development of surface coatings or modifications to reduce microbial adhesion on ceramic and composite resin brackets could significantly improve their clinical performance (12,13).

Despite the strengths of this study, including the standardized in vitro methodology, certain limitations must be acknowledged. The study did not account for variations in oral conditions, such as salivary flow and dietary habits, which can influence biofilm formation in vivo. Additionally, the use of a single bacterial strain may not fully replicate the complexity of the oral microbiome. Future research should explore the effects of multispecies biofilms and in vivo conditions to validate these findings (14,15).

CONCLUSION

In conclusion, the study highlights the influence of bracket material on biofilm formation, with metallic brackets being the least biofilm-retentive. These findings underscore the need for material selection and surface modification strategies to minimize biofilm-related complications in orthodontic patients.

Conflict of Interest: None

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Authors contributions:

H.A.; J.M.G., S.T.K., M.K.A.; conceived the research idea; H.A.; M.K.A.; prepared the article; H.A.; M.K.A. collected and tabulated the information; H.A.; J.M.G., S.T.K., M.K.A. carried out the bibliographic search; H.A.; J.M.G., S.T.K., M.K.A. helped in the development of the discussion; and H.A.; J.M.G., S.T.K., M.K.A. carried out the critical revision of the article. All authors approved the final version of the article.



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