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Streptococcus mutans Adhesion: A Controlled In vitro Investigation Using Bracket and Composite Material in Relation to Saliva pH

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Abstract:

Background: The risk of caries is increased when Streptococcus mutans adheres to orthodontic equipment, which leads to the production of biofilms. The purpose of this research was to determine the in vitro effects of bracket material, composite type, and saliva pH on S. mutans adhesion. The following procedures were used to evaluate six different combinations of brackets and composites: plastic, metal, ceramic brackets with light-cure and self-cure composites, and artificial saliva with a pH of 5 and a pH of 7, respectively. Six samples were produced for each of the twelve groupings. Following the development of the pellicle, the samples were exposed to 1.5×10^1 CFU/mL of S. mutans and the number of colony-forming units (CFUs) was measured. With a significance level of 0.05, the data were examined using generalized estimating equations (GEEs). The results showed that at a pH of 5, the average CFU/mL for metal brackets was 59.10 ± 5.23, 93.11 ± 83.25, and for ceramic brackets it was 87.77 ± 35.61. Two kinds of composites were produced: 72.18 ± 67.92 for light-cure and 308.93 ± 75.19 for self-cure. A significant main impact of bracket material (P = 0.001) and an interaction between brackets and composites (P = 0.001) were seen when the two variables were combined, however GEE did not find any significant effects of either bracket material (P = 0.183) or composite type (P = 0.966) when examined separately. The averages for ceramic, metal, and plastic brackets at pH 7 were 816.38 ± 50.72, 393.32 ± 67.50, and 671.41 ± 83.25 CFU/mL, respectively. Light-cure and self-cure composites demonstrated 84.33 ± 78.94 and 243.22 ± 67.43 CFU/mL, respectively. According to GEE, there was no interaction (P = 0.234), a composite effect (P = 0.001), and a bracket effect (P = 0.095) that was not statistically significant. The results show that the saliva pH controls the material impacts on the adherence of S. mutans. Adhesion differences are driven by bracket-composite interactions in acidic circumstances, whereas the composite type predominates at neutral pH. The oral pH environment and material matching should be considered while selecting an appliance.

Staphylococcus mutans, dental materials, orthodontic equipment, saliva, and bacterial adherence are all important concepts to consider.

INTRODUCTION

Streptococcus species are among the many oral bacteria found in the mouth; these bacteria are thought to be responsible for dental caries and gum disease. [1] An essential part of biofilm growth is the adhesion of Streptococcus mutans to orthodontic device surfaces. This biofilm may continue to grow even after regular chemical and mechanical cleanings, and it causes demineralization of the enamel and irritation of the gingiva during orthodontic treatment. Various in vitro studies have investigated the effects of bracket material on S. mutans adherence. According to Kato et al., ceramic brackets showed a substantial decrease in biofilm. Evidence suggests that orthodontic composite bonding agent type, in addition to bracket material, influences plaque buildup. medical care. [2,3] Plaque growth around brackets caused by bacteria is in particular, as it greatly raises the possibility that patients receiving fixed appliance treatment would develop white-spot lesions and interproximal caries. 2, 4

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It is probable that variations in surface free energy and density of polymer networks explain why Yassaei et al. found that S. mutans adhered more strongly to self-cure composites than to light-cure variations. Based on their evaluation of several surface coatings on both kinds of composites, Tavakolinejad et al. [8] verified that some treatments may significantly decrease bacterial colonization on bracket-composite assemblies. [9] An other important factor that determines bacterial adhesion is the environmental pH. S. mutans is more electrostatically attracted to the device when the pH is acidic (about 5), since the net surface charge of the brackets changes. The study by Math et al. revealed that, in comparison to neutral pH (pH 7), acidic saliva simulants enhanced surface binding of S. mutans across all kinds of brackets, with colony numbers rising by as much as 25%. [10] Under pH 5 settings, Mirmohammadi et al. confirmed these results by showing that bacterial adherence on plastic and ceramic surfaces increases by 15%-20%. the eleventh

A significant void exists in the literature, despite the abundance of single-factor and dual-factor investigations: no study has yet evaluated three bracket materials (plastic, metal, and ceramic) with two kinds of composites (light-cure and self-cure) across two saliva pH values (5 and 7). The majority of the available literature focuses on a single bracket-composite system, either on the material or type of bracket alone, or on variations in pH. Numbers 5–11. To provide evidence-based guidelines for clinical orthodontic appliance selection and oral hygiene, it is necessary to fill this three-part gap.

Therefore, the purpose of this in vitro research is to examine the adherence of S. mutans across metal, plastic, and ceramic bracket substrates bonded with light-cure or self-cure composites under saliva simulators at pH 5 and pH 7 utilizing colony-forming unit (CFU) counts.

METHODS

Ethical consideration

The study was approved by the Research Ethics Committees of Islamic Azad University– Dental Branch Tehran-Iran (IR. IAU.DENTAL.REC.1402.048). Collection of the samples and processing were done as per the Helsinki Declaration, 2013.

Type of sampling and reasons for selection

This *in vitro* study was designed and conducted to evaluate the effects of bracket material, composite type, and artificial saliva pH on the adhesion of *S. mutans*. The study samples comprised plastic, metal, and ceramic orthodontic brackets bonded with either light-cure or self-cure composites, each exposed to artificial saliva at pH 5 and pH 7 under *in vitro* conditions.

Based on a power analysis conducted in PASS v. 11 using a fixed-effect one-way ANOVA, the following parameters were set: $\alpha = 0.05$, $\beta = 0.01$ (power = 0.99), and effect size (Cohen's f) = 1.5 for the "caries risk" variable. This yielded a minimum of six samples per subgroup. Samples were randomly assigned to the six bracket–composite combinations [Table 1].

Streptococcus mutans preparation

S. mutans UA159 (ATCC 700610) was obtained from the National Genetic and Biological Resource Center of Iran. The strain was incubated in Brain–Heart infusion (BHI) broth supplemented with 1% glucose at 37°C under 10% CO_2 for 24–48 h. Cells were pelleted by centrifugation at 3000 × *g* for 10 min, washed twice with sterile 145 mM NaCl, and resuspended in the same to a final concentration of 1.5 × 10^9 CFU/mL, confirmed by OD_{660} measurements.

Artificial saliva preparation

Phosphate-buffered saline (PBS, pH 7) was used as the control, and acidic artificial saliva (pH 5) was prepared using: NaCl 400 mg/L, KCl 400 mg/L, $CaCl_2 \cdot 2H_2O$ 795 mg/L, $NaH_2PO_4 \cdot H_2O$ 690 mg/L, $Na_2S \cdot 9H_2O$ 5 mg/L, and urea (CH_4N_2O) 1000 mg/L. The six bracket–composite combinations were tested at each pH level. Six samples ($n = 6$) per combination were randomly selected and incubated in PBS or acidic saliva for a total of 72 samples: (1) Ceramic brackets (3 mm diameter, 12 mm thickness); (2) Stainless steel brackets (3 mm diameter, 12 mm thickness); (3) Plastic brackets (OK Real Resin, 3 mm diameter, 12 mm thickness); (4) Light-cure composite (3M™ Transbond™ XT); and (5) Self-cure composite (Vertise™ Flow).

Composite sample preparation

For each bracket–composite combination at each pH, six resin blocks (4 mm × 4 mm × 1 mm) were fabricated (72 blocks total) using a Teflon mold and polished with 1,000-and 2,000-grit sandpaper. Composite resin was packed into the mold and polymerized with an Ortholux light-curing unit (3M Unitek) at 1200 mW/cm² for 60 s. Blocks were washed in an ultrasonic bath (Mini Sono Cleaner CA-1470) for 15 min in deionized water, then 30 min in 70% ethanol, and sterilized with ethylene oxide gas.

Table 1: Bracket-composite material combinations evaluated in the study

Combination	Bracket material	Composite type
1	Ceramic	Light-cure
2	Ceramic	Self-cure
3	Metal	Light-cure
4	Metal	Self-cure
5	Plastic	Light-cure
6	Plastic	Self-cure

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Sterilized blocks were immersed in artificial saliva (pH 5 or 7) for 2 h to form an acquired pellicle, then incubated for 2 h in *S. mutans* suspension (1.5×10^9 CFU/mL) in BHI. After incubation, blocks were rinsed three times with 500 µL of 0.9% NaCl to remove nonadherent bacteria, then sonicated at 50% amplitude (Qsonica 125) for 3 s in 10 mL saline to detach adherent bacteria. The resulting suspensions were serially diluted, and 10 µL droplets of each dilution were plated in triplicate on BHI agar. Plates were incubated at 37°C ± 1°C for 48 h, after which CFUs were counted. For high-density plates, dishes were divided into equal sectors, and the average CFU per sector was counted, and multiplied by the number of sectors to obtain total CFU.

Patient consent statement

This research was not done on human samples but on synthetic samples.

Inclusion criteria

Resin composite blocks (light-cure and self-cure) fabricated using a Teflon mold (4 mm × 4 mm × 1 mm) and polymerized at 1200 mW/cm² for 60s.

Standard plastic, metal, and ceramic brackets (3 mm diameter, 12 mm thickness) polished with 1000- and 2000-grit sandpaper and sterilized with ethylene oxide.

Formation of an acquired pellicle by immersing samples for 2 h in PBS (pH 7) or acidic artificial saliva (pH 5).

Use of *S. mutans* UA159 at a final concentration of 1.5×10^9 CFU/mL with a 2-h incubation in BHI broth.

Availability of CFU count data for each sample in three independent replicates.

Exclusion criteria

Blocks or brackets exhibiting mechanical damage or contamination during polymerization, polishing, or sterilization.

Samples with unsuccessful pellicle formation or incomplete bacterial incubation (failure to reach the target CFU/mL).

Use of *S. mutans* strains other than UA159 or bacterial concentrations different from 1.5×10^9 CFU/mL.

Samples lacking complete CFU data (fewer than three replicates or inconsistent serial dilutions).

Exposure to conditions deviating from the defined protocol (37°C, 5% CO_2, pH 5 or 7).

Data availability statement

Two main methods were used to collect data; Direct Observation: After incubation, bracket–composite samples were examined under a light microscope to assess biofilm distribution. Quantitative Recording and CFU Counting: Bacterial colony counts (CFU) were recorded using standardized forms and determined by culture and colony enumeration techniques.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Statistical analysis

Data were organized in Microsoft Excel and transferred to IBM SPSS-26 Statistics (Armonk, New York, USA) for analysis. A generalized estimating equations (GEEs) model was used to assess the effects of bracket material, composite type, and saliva pH on *S. mutans* CFU counts, with a significance level of $\alpha = 0.05$. Results are presented as mean ± standard deviation.

RESULTS

Table 2 presents the results of the GEEs analysis under acidic saliva conditions (pH = 5). The *P* values for all comparisons exceeded 0.05, indicating no statistically significant differences in *S. mutans* adhesion levels across the three bracket materials. Plastic brackets served as the reference category. While ceramic and metal brackets showed slightly lower mean CFU counts compared to plastic brackets, these differences were not statistically significant. Although plastic brackets showed the highest mean CFU count, the lack of statistical significance suggests that, under acidic conditions, bracket material alone does not significantly affect bacterial adhesion.

Table 3 presents the results of the GEEs analysis comparing the adhesion of *S. mutans* to light-cure and self-cure composites under acidic conditions (pH = 5). The *P* value exceeded 0.05, indicating no statistically significant difference in bacterial adhesion between the two composite types

Self-cure composite was used as the reference category. Although light-cure composite showed a slightly lower mean CFU count, this difference was not statistically significant.

These results suggest that under acidic pH conditions, the type of orthodontic composite (light-cure vs. self-cure) does not significantly influence *S. mutans* adhesion.

According to the GEE analysis summarized in Table 4, statistically significant effects (denoted by an asterisk) were observed at the 0.05 level. Specifically: The main effect of bracket material was statistically significant ($P = 0.001$); The main effect of composite type was not statistically significant ($P = 0.118$), but the interaction between

Table 2: Generalized estimating equations results for bacterial adhesion to bracket materials at pH 5

Bracket material	CFU/mL, mean±SD	Test statistic	*P*
Ceramic	87.77±35.61	1.769	0.183
Metal	59.10±5.23	0.019	0.891
Plastic (reference)	93.11±83.25	-	-

SD: Standard deviation, CFU: Colony-forming units

Table 3: Generalized estimating equations results for bacterial adhesion to composite types at pH 5

Composite type	CFU/mL, mean±SD	Test statistic	*P*
Light-cure	72.18±67.92	0.002	0.966
Self-cure (reference)	308.93±75.19	-	-

SD: Standard deviation, CFU: Colony-forming units

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bracket material and composite type was statistically significant ($P = 0.001$).

Overall Interpretation (at pH 5)

At pH 5, the amount of S. mutans adhesion to acquired pellicles on metal, plastic, and ceramic brackets varies greatly depending on the type of bracket. Light-cure and self-cure orthodontic composites did not differ significantly in S. mutans adhesion at this pH level. A significant interaction was observed between bracket material and composite type, suggesting that the magnitude of one variable's effect is dependent on the other. That is to say, when examined separately at pH 5, there was no discernible difference in bracket types. However, when looking at the data as a whole, the GEE analysis showed that there was a main effect of bracket material (P = 0.001) and an interaction between bracket type and composite type. It seems that the kind of composite employed interacts with the bracket material to affect S. mutans adherence. All of the bracket materials had P values more than 0.05, according to the GEEs analysis findings at neutral pH [Table 5]. Thus, it seems that at pH 7, there was no significant difference in the attachment of S. mutans to acquired pellicles generated on plastic, metal, and ceramic brackets. The GEE investigation comparing the adherence of S. mutans to light-cure and self-cure composites under neutral circumstances (pH 7) is summarized in Table 6. The two composite kinds did not vary significantly at this pH, since all P values were more than 0.05. These results validate that, in neutral saliva circumstances (pH 7), there is no significant difference in S. mutans adherence influenced by light-cure or self-cure orthodontic composites. Only the main impact of composite type achieved statistical significance according to the GEE analysis findings at neutral saliva pH [Table 7], however the interaction between bracket material and composite and the composite itself did not. Conclusion (at pH 7) At pH 7, there was no discernible variation in the adherence of S. mutans to plastic, metal, and ceramic brackets. On the other hand, a notable disparity was noted between light-cure and self-cure composites (P = 0.001), suggesting that the type of composite alone affects bacterial adhesion in neutral conditions. Lastly, no significant interaction between the bracket material and the composite type was found, indicating that the impact of either factor is independent of the other at pH 7.

Table 4: Generalized estimating equations analysis of main and interaction effects at pH 5

Variable	Test statistic	P
Main effects		
Bracket material	168.686	0.001*
Composite type	2.443	0.118
Interaction effects		
Bracket material × composite type	22.287	0.001*

*Significance level $p < 0.05$

Table 5: Generalized estimating equations results for bacterial adhesion to bracket materials at pH 7

Bracket material	CFU/mL, mean±SD	Test statistic	P
Ceramic	816.38±50.72	0.038	0.845
Metal	393.32±67.50	0.373	0.541
Plastic (reference)	671.41±83.25	-	-

SD: Standard deviation, CFU: Colony-forming units

Table 6: Generalized estimating equations results for bacterial adhesion to composite types at pH 7

Composite type	CFU/mL, mean±SD	Test statistic	P
Light-cure	84.33±78.94	1.5	0.221
Self-cure (reference)	243.22±67.43	-	-

SD: Standard deviation, CFU: Colony-forming units

Table 7: Generalized estimating equations analysis of main and interaction effects at pH 7

Variable	Test statistic	P
Main effects		
Bracket material	4.706	0.095
Composite type	12.593	0.001*
Interaction effects		
Bracket material × composite type	2.902	0.234

*Significance level $p < 0.05$

DISCUSSION

In this *in vitro* investigation, we assessed the adhesion of *S. mutans* to acquired pellicles on six bracket–composite combinations under acidic (pH 5) and neutral (pH 7) saliva conditions. Under acidic conditions, independent analyses showed no significant differences in bacterial adhesion among bracket materials or composite types; however, the full GEE model revealed both a significant main effect of bracket material ($P = 0.001$) and a significant bracket × composite interaction ($P = 0.001$). This finding aligns with Kato *et al*.,[5] who reported that ceramic brackets exhibit lower biofilm formation compared to metal and plastic counterparts due to differences in surface roughness and wettability, and with Hasanzadeh Azhiri *et al*.,[7] who demonstrated that the crystalline structure of ceramic brackets modulates surface hydrophilicity and bacterial attachment depending on the contacting resin matrix. It also helps explain why Noorbakhsh *et al*.[6] observed higher *S. mutans* counts on plastic brackets – their inherently rougher surfaces – but only in certain composite pairings does this translate into a statistically significant increase.

Supporting the clinical results of Ma et al., who found no significant difference in S. mutans numbers close to brackets bonded with resin-modified glass ionomer cement vs resin-based composite in a neutral environment, the independent effect of bracket material on S. mutans adherence was also not seen.

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experimental design (P > 0.05). [12] However, at pH 7, there was a significant effect of composite type (P = 0.001), with self-cure resins exhibiting greater bacterial adhesion than light-cure ones. This finding is in line with Yassaei et al., [8] who also discovered no significant difference between light-cure and self-cure resins under acidic challenge. Duraisamy et al.[14] showed that hybrid nano-coatings may attenuate these disparities, while Feres et al.[13] connected reduced polymer crosslink density in self-cure composites to greater microbial adhesion under neutral pH. Our observation that the effects of isolated materials or resins vanish at pH 5 but return at pH 7 is consistent with the dominating function of pH in regulating electrostatic interactions, as shown by Math et al.[10] and Mirmohammadi et al.[11] as improved S. mutans binding in acidic environments.
Clinically, these results indicate that the ideal choice of bracket and composite material should be based on the patient's oral pH. Light-cure composites work best in neutral environments, but surface treatments like silver or hybrid nano-coatings[15,16] and careful bracket polishing[17] may be necessary to prevent biofilm formation in acidic conditions, which are common in patients with low salivary flow. In conclusion, our work highlights the significance of conducting orthodontic appliance selection and hygiene procedure evaluations based on evidence by simulating real-life saliva settings to assess bracket-composite interactions.

CONCLUSION

This well-controlled in vitro study proved that the effect of orthodontic device materials on S. mutans adherence is significantly affected by saliva pH. Bracket material and composite type did not individually affect bacterial attachment under acidic circumstances (pH 5), but they did interact considerably, suggesting that certain bracket-composite combinations might increase or decrease adhesion. Bracket material had no effect in neutral saliva (pH 7), while self-cure composites allowed considerably more S. mutans colonization than light-cure alternatives, and no interaction effects were seen. The importance of choosing bracket and composite combinations that are specific to each patient's oral pH environment is highlighted by these results. Patients prone to acidic oral conditions may benefit from tailored surface treatments, such as nano-coatings or customized polishing methods, to decrease biofilm development. Light-cure composites may be preferred for persons with normal saliva pH. To further decrease microbial adherence on fixed orthodontic equipment, future research should examine other surface alterations and investigate long-term therapeutic effects.
Results from the research
Findings from this research highlight the importance of material choice and oral pH in determining S. mutans adherence to orthodontic products. When exposed to acidic environments (pH 5), the combination of bracket type and composite had no discernible effect.

bacteria to adhere, but the fact that they interacted so much suggests that certain combinations of brackets and composites might accelerate or slow down the process of biofilm development. On the other hand, without any interaction, self-cure composites supported a much higher amount of S. mutans adherence than light-cure ones, whereas bracket material had no influence at neutral pH. The results show that the right appliance should be chosen based on the patient's salivary pH and the adhesive and bracket qualities taken together.
The study's rationale
No previous study has simultaneously examined all three variables—bracket material, composite type, and saliva pH—that impact S. mutans adherence, despite substantial research on individual aspects. The success of orthodontic therapy and the prevention of dental caries depend on controlling biofilm, but little is known about how different device materials interact with the ever-changing oral environment. This study addresses that knowledge gap by methodically testing the performance of bracket-composite combinations in acidic and neutral saliva simulators. The results will help with material selection and clinical protocols to reduce microbial colonization during fixed-appliance therapy.
Study limitations
Due to its limitations—including its single S. mutans strain, two pH levels, and six bracket-composite combinations—this in vitro investigation may not accurately portray the complicated oral environment. Mechanical stresses, variations in saliva flow, multi-species biofilms, and long-term biofilm maturation were not included in the simulation.

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