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Adhesion of *Streptococcus mutans* to Different Types of Brackets

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ABSTRACT

Objective: To examine the difference in the adhesion of *Streptococcus mutans* to three different types of orthodontic brackets and the effect of the presence of an early salivary pellicle and *Streptococcus sanguis* on adhesion.

Materials and Methods: Three adhesion experiments were performed using stainless steel, ceramic, and plastic orthodontic brackets. In the first experiment a clinical strain of *S mutans* adhered to the three different types of brackets (n = 6 for each). For the second, the brackets were treated with saliva before adhesion of *S mutans* (n = 6 per type of bracket). Finally, the third experiment concerned saliva coated brackets (n = 6 per type of bracket), but before *S mutans*, *S sanguis* bacteria were allowed to adhere. The bacteria were always allowed to adhere for 90 minutes in all the experiments. Adhesion was quantitated by a microbial culture technique by treating the brackets with adhering bacteria with trypsin and enumerating the total viable counts of bacteria recovered after cultivation.

Results: There were consistently no differences in the adherence to stainless steel, ceramic, or plastic brackets. The presence of an early salivary pellicle and *S sanguis* reduced the number of adhering *S mutans* to all three types of brackets.

Conclusions: Adhesion of bacteria to orthodontic brackets depends on several factors. The presence of a salivary pellicle and other bacterial species seem to have a significant effect on the adhesion of *S mutans*, reducing their numbers and further limiting any differences between types of brackets.

KEY WORDS: Orthodontic brackets, Bacterial adhesion, Salivary pellicle, *Streptococcus mutans*.

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INTRODUCTION Return to TOC

The dental literature suggests that orthodontic treatment with fixed appliances leads to an increased plaque accumulation[1] and elevated levels of mutans streptococci and lactobacilli,[2,3] which are considered the main pathogens in dental caries. This can increase the risk of decalcification, which can involve up to 50% of patients, and can lead to the development of caries.

Prevention of these lesions is an important concern for orthodontists. Furthermore, the bracket material could play a role in the degree of bacterial adhesion and plaque accumulation as well as in the risk of caries development. The initial affinity of bacteria to solid surfaces is due mostly to electrostatic and hydrophobic interactions. Surfaces with high surface free energy more easily attract bacteria such as *S mutans*.[4] In a study by Eliades et al[5] stainless steel presented the highest critical surface tension and can be expected to have a higher plaque retaining capacity.

Metallic orthodontic brackets have been found to induce specific changes in the oral environment such as reduced levels of pH, increased plaque accumulation, and elevated *S mutans* colonization. Nevertheless recent studies on possible differences in the initial affinity and adherence of bacteria on metal, ceramic, and plastic brackets over time were inconclusive.[6,7] Therefore, it is difficult to make a clear assessment that metal brackets have a lower cariogenic effect on the teeth than plastic or ceramic brackets.

An additional important factor for microbial colonization of oral hard surfaces is the salivary or acquired pellicle which can form not only on tooth surfaces, but also on restorations, and prosthetic and orthodontic appliances. Therefore, the adhesion of oral microorganisms to the bracket surface is influenced to a large extent by interactions between salivary components in the pellicle and properties of the different microorganisms, in addition to the adherent patterns of bacteria on the different types of orthodontic brackets.[7]

The aims of the present study were to: (a) investigate the adhesion of *S mutans* to stainless steel, plastic, and ceramic brackets with/without an acquired pellicle and (b) evaluate the adhesion of *S mutans* to these three types of brackets in the presence of *Streptococcus sanguis*.

MATERIALS AND METHODS Return to TOC

Bacteria Sampling and Culture Procedures

S mutans and *S sanguis* were isolated from pooled plaque samples which were taken by sterile wooden wedges from the interproximal area between the first and second primary molars from each quadrant (4 wedges per patient) of a child with active caries. The wedges were dispersed in 1 mL reduced transport fluid (RTF) and prepared for further microbiological processing within 3 hours. Serial 10-fold dilutions were prepared and inoculated on TYCSB (trypticase yeast cysteine sucrose Bacto Agar) selective growth media for *S mutans*[8] and on

nonselective ETSA (enriched trypticase soy agar) plates[9] for the isolation of *S sanguis*. The inoculated plates were incubated for 3 days in 10% CO_2 at 37°C. The presumptive characterization and identification of *S mutans* and *S sanguis* were based upon the colony morphology, Gram stain, and catalase activity, while the definitive identification was made with the API Strep 20 system (Biomerieux SA, Montalieu-Vercieu, France). The identified bacteria were stored in sterile vials containing porous beads (Microbank, Pro Lab Diagnostics) at −70°C.

Before the bacterial adhesion experiments to brackets, a few beads were taken with a sterile microbiological loop from the frozen cultures of *S mutans* and *S sanguis* and were individually spread on blood agar plates (Blood Agar Base II; Oxoid, Basingstoke, England) supplemented with hemin (5 µg/mL), menadione (1 µg/mL), and 5% sterile horse blood, and incubated for 3 days in jars (5% CO_2) at 37°C. From these colonies, pure cultures were prepared on hard blood agar plates, further supplemented with 0.8% (w/v) Bacto Agar (Difco Laboratories, Detroit, MI). The latter increased the hardness of the agar plates. The harder plates ensured that a minimal, if any, amount of agar was scraped off with the cotton tips. The bacterial concentration was adjusted by optical density measurements based on a previously calculated optical density/bacterial concentration gradient curve. After 48 hours they were collected and suspended in sterile phosphate-buffered saline (PBS) solution for the adhesion assays.

Preparation of Early Salivary Pellicle

At the day of the experiment of adhesion, saliva was selected from two healthy 40-year-old adults. They had not taken any medication during 3 months before the study and had no active caries or periodontal disease. Stimulated saliva was collected by chewing paraffin gum for 5 minutes and expectorating into a sterile plastic cup. The saliva was immediately clarified by centrifugation at 12,000 g for 20 minutes at 4°C and filtered using cellulose acetate membrane filters (pore size 0.22 µm).[10]

For the formation of an early salivary pellicle, the brackets used were placed into Costar 24-well culture plates (Corning, Corning, NY), and 1 mL of saliva was added to each well. They were incubated for 30 minutes at 37°C, after which they were removed and placed in new 24-well plates for the adhesion assays.

Adhesion of Bacteria to Orthodontic Brackets

Metallic (stainless steel), ceramic (polycrystalline alumina), and plastic (polycarbonate) maxillary central incisor brackets (American Orthodontics, Sheboygan, WI) were included in the study. All brackets had a 0.018-inch slot. The bacteria used for all the adhesion assays were always from the same isolated clinical strains, for both species.

Part I: Effect of Bracket Type

In a first experiment, six brackets of each type were placed in individual wells of a Costar 24-well culture plate. A 2-mL PBS suspension of approximately 10^8 per mL *S mutans* was added to each well. The brackets with the bacterial suspension were aerobically incubated at 37°C for 90 minutes, with intermittent shaking. Afterwards, the brackets were rinsed 2× carefully with PBS to remove any nonadherent bacteria.

Part II: Effect of Early Salivary Pellicle

In order to examine the effect of the salivary pellicle on the adhesion of *S mutans*, six brackets of each three types (stainless steel, ceramic, and plastic) were prepared with saliva as mentioned above. After the formation of the pellicle, a 2-mL PBS suspension of approximately 10^8 per mL *S mutans* was added to each well. The brackets with the bacterial suspension were aerobically incubated at 37°C for 90 minutes, with intermittent shaking. Afterwards, the brackets were rinsed 2× carefully with PBS to remove any nonadherent bacteria.

Part III: Effect of the Presence of *S sanguis*

For the role *S sanguis* may play in the adhesion of *S mutans* the two species were allowed to adhere to the same brackets, sequentially. Six brackets of each type (stainless steel, plastic, and ceramic) were prepared with saliva, as outlined above, in order for a salivary pellicle to form. First, *S sanguis* was allowed to adhere for 1½ hours. The brackets were rinsed 2× carefully with PBS to remove any nonadherent bacteria, and then *S mutans* was allowed to adhere for 1½ hours. The PBS suspensions consisted of 2 mL with approximately 10^8/mL *S mutans* and 2 mL with approximately 10^8/mL *S sanguis* for each well. The brackets with each of the bacterial suspensions were aerobically incubated at 37°C for each 90-minute period, with intermittent shaking. Afterwards, the brackets were rinsed 2× carefully with PBS to remove any nonadherent bacteria.

Culture of Adhering Bacteria

For each experiment, after the washing with PBS, the brackets with their adhering bacteria from each plastic well were treated with 2 mL of 0.25% trypsin/ EDTA for 45 minutes in aerobic conditions at 37°C, for detachment of the adherent bacteria. Serial dilutions were prepared after thorough pipetting and vortexing the initial solution. The dilutions were then plated by hand onto blood agar plates. For each bacterial species, serial dilutions of the initial concentration were also plated to control the number of bacteria added to each well. After 4 days of incubation in jars (5% CO_2) at 37°C, the total number of viable counts (TVC)/well was determined. The unit of adhesion was considered to be the colony unit formed.

For Part III, where two bacterial species were involved, the isolated bacteria were characterized and identified based upon the colony morphology, Gram stain, and catalase activity, while the definitive identification of the isolates was made with the API Strep 20 system.

Statistical Analysis

All statistical analyses were performed using the data analysis toolkit of Microsoft Office Excel 2003 (Richmond, WA). One-way analysis of variance (ANOVA) was used for Part I, where only the effect of the bracket type was examined. Two-way ANOVA was used for the other two parts. Total number of adherent bacteria (as represented by the TVC) per type of bracket was tested. For all analyses $P < .05$ was considered statistically significant.

RESULTS

Part I: Effect of Bracket Type

In the first experiment for the noncoated brackets, no significant differences were found in the adhesion of *S mutans* on the different types of brackets. A total of 2.66×10^5, 2.95×10^5, and 4.23×10^5 bacteria were found for the stainless steel, ceramic, and plastic brackets, respectively (Figure 1).

Part II: Effect of Salivary Pellicle

The adhesion of *S mutans* to saliva coated brackets was significantly lower (Table 1) than the adhesion to non–saliva coated brackets ($P < .0001$). This difference consistently surpassed a log score for all three of the different bracket types (stainless steel, ceramic, and plastic), while again the type of bracket had no significant effect on bacterial adhesion (Table 1b).

Part III: Effect of the Presence of *S sanguis*

When the adhesion of *S mutans* on different types of brackets was tested, in the presence of *S sanguis* that had been allowed to previously adhere, results again showed very significant differences ($P < .0001$) (Table 2b). More bacteria of *S mutans* could adhere to the brackets when they were tested alone than when *S sanguis* was added (Table 2a).

The pattern of adhesion was similar for all three types of brackets tested and no significant surface effect was detected.

DISCUSSION

The molecular and elemental composition of biomaterial surfaces is considered the determinant factor of the early response of the biological environment. The polar or nonpolar nature, the hydrogen bonding capacity, and the electron donor or acceptor potential, seem to control the hydrophilic or hydrophobic character and the energetic state of the surfaces. In addition, surface electrical properties, such as the zeta and streaming potentials and surface charges, are also involved in interfacial interactions with biological fluids and living cells.

In the orthodontic bracket surface exposed to oral environment, initially, on a nanosecond scale, a water monolayer binds to a biomaterial surface by either oxygen or hydrogen bonding. Some water molecules may dissociate to hydroxyl groups, which may form surface hydroxyls. Then, a second water layer binds to the first monolayer. The orientation and density of water molecules in the first adsorbed monolayer may regulate the overall hydration state of the surface, because the unique properties of water offer a wide spectrum of solvation forces in aqueous systems.[11]

On hydrophilic surfaces, for example, to which water molecules bind strongly, repulsive hydration (long-range) and steric (short-range) forces are generated when two such surfaces come in contact because of the energy required to dehydrate the surfaces; these forces are controlled by the presence of cations or pH. On hydrophobic surfaces on the other hand, the orientation of water molecules towards the surfaces is entropically unfavorable.[12] Thus, in the event that two such surfaces approach each other, water is ejected into the bulk solution, reducing the total free energy of the system and establishing attractive long-range hydrophobic forces between the two surfaces. These phenomena are of paramount importance for biological systems and control protein adsorption, which proceeds by nonspecific physicochemical interactions with the water- and ion-modified biomaterial surfaces.

In an aqueous environment, the interaction between hydrophilic surfaces with strongly attached water molecules and hydrated protein cores is weak, due to the development of hydrophilic repulsive forces. Thus, on hydrated surfaces, hydrophobic and electrostatic interactions are expected to govern protein adsorption. In most situations competitive adsorption occurs, with rapidly decreasing protein affinity accompanying increasing surface occupancy. The result is a sequential adsorption/desorption process and exchange of proteins on surfaces, a phenomenon known as the Vroman effect. The adsorption/desorption sequence of proteins is not distinct since all proteins are adsorbed simultaneously at different rates, and displacement occurs according to their binding affinity; the latter is associated with the spreading capacity and the energy required to denature the proteins in the solution.[13,14]

Our findings indicate that saliva is an important factor in the adhesion of *S mutans* to orthodontic brackets. Presence of saliva and the formation of an early salivary pellicle decrease the adhesion of bacteria on the contrary with the non–saliva coated brackets. These findings are in agreement with those from other studies.[6,7] The 30 minutes that the pellicle was allowed to form was enough to affect the adhesion of the bacteria examined (comparably to the aforementioned studies[6,7] in which more mature [2-hour] pellicles were used). This may be explained by the fact that saliva-coating reduces the surface free energy of the underlying materials,[15] changes found to occur even within 30 minutes of pellicle formation.[5] Additionally, the presence of histatins and lyzozymes in saliva which possess exceptional antibacterial activities, may also contribute to the decreased adhesion of *S mutans* to brackets.

In noncoated samples, only the bracket surface characteristics can affect adhesion of bacteria. Therefore, bacteria with high surface–free energy such as *S mutans*[16] should prefer surfaces with high surface–free energy materials such as stainless steel brackets.[5] Previous studies have shown conflicting results. Fournier et al[6] found that *S mutans* had a significantly lower affinity to stainless steel compared to plastic and porcelain brackets, while another study, by Ahn et al,[7] found that stainless steel had the highest.

However, although slight differences were found in the adhesion of *S mutans* to different types of brackets (with the lowest adhesion recorded for stainless steel) in the present study, these did not reach a level of statistical significance. Direct comparisons of results, however, between the different studies must be made with care and must take into consideration the different methodologies used to examine this interaction between hard surfaces and bacteria. The most important factor that may explain the differences of the present study with the previous is the fact that live cultures were used to examine the number of adhering bacteria, whereas the others used radiolabelled bacteria and measurements of radioactivity.

The fact that the present study showed no differences in the adhesion capability of bacteria to the different bracket types, combined with the important role that the salivary pellicle may play, negating any differences in surface characteristics, may indicate that no specific bracket may have a lower cariogenic effect on the teeth than the other. However, an additional factor that may play a role in experiments with adhesion (and especially in clinical situations) is the size and different shapes of available brackets that may provide retentive surfaces for the formation of dental plaque. Even the amount of time that the bacteria have available to adhere may affect the results. By increasing the incubation time, adherence of *S mutans* has been shown to increase.[17] However, for this factor too the opposite has been shown, with a decrease in the affinity of bacteria to the bracket surfaces over time.[6]

In the presence of *S sanguis*, the adhesion of *S mutans* to brackets was significantly lower (*P* < .0001) than the adhesion of the bacteria alone. *S sanguis* seems to have an antagonistic relationship with *S mutans* as far as adhesion and colonization are concerned.[18] In the presence of *S sanguis*, the binding sites of *S mutans* on the salivary pellicle formed on the bracket are reduced. Our results would thus seem to be consistent with the theory that *S sanguis* acts as an antagonistic bacterium. A delayed colonization by mutans streptococci may lead to less caries or susceptibility to caries.[19] The adhesion of bacteria on brackets would seem to be more complicated, in a situation like the oral cavity where interactions between the salivary pellicle, many different bacteria, and bracket's surface characteristics take place, than the one examined in vitro. These factors should always be kept in mind when performing adhesion experiments, whether it is brackets or other material.

Future clinical studies of the oral health and microflora between patients wearing different types of brackets would help determine any difference of clinical importance in the plaque composition and the cariogenic effect of each type of bracket on the oral health of the orthodontic patient.

CONCLUSIONS

- No obvious difference was found in the adhesion of *S mutans*, whether they were alone or in presence of *S sanguis*, to stainless steel, plastic, and ceramic orthodontic brackets.

- Saliva and more specifically the salivary pellicle play an important role in the adhesion of bacteria, reducing the number of adhering *S mutans*.

- *S sanguis* seems to have an antagonistic relationship with *S mutans*, interfering with its adhesion.

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TABLES

Table 1. **Table 1a.** Results for Part II: The Effect of the Salivary Pellicle Mean Number and the Standard Deviation (SD) of the Total Adhering Bacteria Per Type of Bracket With and Without the Presence of a Salivary Pellicle

	Stainless Steel		Ceramic		Plastic	
	No Pellicle (n = 6)	Pellicle (n = 6)	No Pellicle (n = 6)	Pellicle (n = 6)	No Pellicle (n = 6)	Pellicle (n = 6)
Mean	2.66×10^5	1.94×10^4	2.95×10^5	1.36×10^4	4.23×10^5	9.20×10^3
SD	1.17×10^5	1.51×10^4	2.20×10^5	9.60×10^3	2.31×10^5	4.53×10^3

Table 1b. Results for Part II: The Effect of the Salivary Pellicle Two-way ANOVA[a] Table for the Effect of the Salivary Pellicle Per Type of Bracket

Source of Variation	SS	dF	MS	F	*P* value	*F* ratio
Effect of pellicle	888200000000	1	8.8824	45.958	<.0001	4.1709
Type of bracket	37440000000	2	18718587778	0.9685	.3912	3.3158
Interactions	46760000000	2	23377521111	1.2096	.3125	3.3158
Within	579800000000	30	19327448444			
Total	1552000000000	35				

[a] ANOVA indicates analysis of variance.

Table 2. **Table 2a.** Results for Part III: Effect of Presence of *S. sanguis* Mean Number and the Standard Deviation (SD) of the Total Adhering Bacteria Per Type of Bracket With and Without the Presence of Adhering *Streptococcus sanguis* (*Ss*)

	Stainless Steel		Ceramic		Plastic	
	Alone (n = 6)	Presence of Ss (n = 6)	Alone (n = 6)	Presence of Ss (n = 6)	Alone (n = 6)	Presence of Ss (n = 6)
Mean	9.68×10^3	1.48×10^3	6.82×10^3	1.51×10^3	4.60×10^3	2.83×10^2
SD	7.56×10^3	1.23×10^3	4.79×10^3	1.34×10^3	2.27×10^3	2.23×10^2

Table 2b. Results for Part III: Effect of Presence of *S. sanguis* Two-way ANOVA[a] Table for the Effect of the Presence of Adhering *Strepto coccus sanguis* (*Ss*) Per Type of Bracket

Source of Variation	SS	dF	MS	F	P value	F ratio
Effect of the presence of *Ss*	317433611	1	317433611	21.500	<.0001	4.1709
Type of bracket	59410555	2	29705277.8	2.0120	.1514	3.3158
Interactions	24457222	2	12228611.1	0.8283	.4465	3.3158
Within	442921667	30	14764055.6			
Total	844223056	35				

[a] ANOVA indicates analysis of variance.

FIGURES
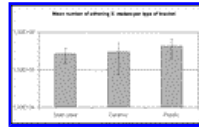
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Figure 1. Mean values for the adhesion of *Streptococcus mutans* to the three different bracket types (n = 6 for each type). No significant difference was detected (*P* = .36)

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