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Presence of cariogenic streptococci on various bracket materials detected by polymerase chain reaction

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Objective: To determine the in vivo presence of *Streptococcus mutans* and *Streptococcus sobrinus* on different bracket materials and to correlate the prevalence with the Visible Plaque Index (VPI) and the Gingival Bleeding Index (GBI).

Methods: Orthodontic brackets made of different materials (stainless steel, gold, ceramic, plastic) were bonded to the upper and lower second premolars in 40 subjects receiving fixed orthodontic appliances. After 30 days, the brackets were debonded and the presence of *S. mutans* and *S. sobrinus* on the brackets was determined using the polymerase chain reaction (PCR) technique. The VPI and GBI were recorded and the relationship between the prevalence of the streptococci and the level of oral hygiene was calculated.

Results: There were fewer *S. mutans* and *S. Sobrinus* over the surface of gold and stainless steel brackets compared with the plastic and ceramic brackets. A statistically significant difference was observed in *S. mutans* and *S. sobrinus* prevalence between the metal brackets and the aesthetic brackets. However, there were no statistically significant differences in *S. mutans* and *S. Sobrinus* prevalence when the gold and stainless steel brackets were compared. Comparison between the plastic and ceramic brackets revealed a similar finding. Furthermore, a significant correlation was found between the in vivo prevalence of *S. mutans* and *S.sobrinus* and the oral hygiene indices ($p < 0.05$), suggesting that the oral hygiene indices could be a good indicator of *S. mutans* and *S. sobrinus* prevalence.

Conclusions: Since microbial adhesion is greater on aesthetic brackets, good oral hygiene during treatment should be emphasised.

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Introduction

Enamel demineralisation or white spot lesions around orthodontic appliances is an unwelcome finding at the end of orthodontic treatment. It is caused by organic acids produced mainly by *Streptococcus mutans* and the closely-related *Streptococcus sobrinus* which are the organisms largely responsible for dental caries.[1,2] These cariogenic streptococci can be isolated and identified by biochemical, immunologic or genetic tests, but the laboratory procedures can be time-consuming and imprecise.[3–5] Recently, the polymerase chain reaction (PCR) has been used to overcome these limitations, as it is possible to amplify a single piece of identifiable, microbial DNA to generate multiple copies.[6]

Orthodontic brackets are made of materials such as stainless steel, ceramic, plastic and gold. The complex design of many brackets provides a unique environment for micro-organisms and impedes cleaning of tooth surfaces adjacent to the bracket base.[7] As a result, the plaque that accumulates around the base can lead to enamel decalcification and gingival inflammation and bleeding. Oral hygiene can be assessed using the Visible Plaque Index (VPI) and gingival bleeding assessed using the Gingival Bleeding Index (GBI).[8,9] Although it is known that the

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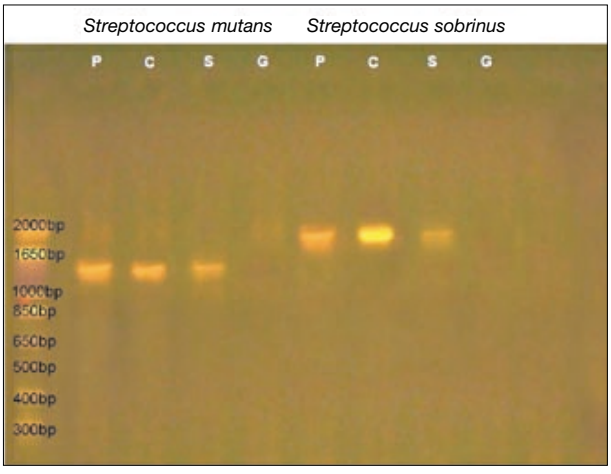


Figure 1. Electrophoretic bands examined with a UV transilluminator.

intra-oral microbial population changes following placement of fixed orthodontic appliances, little information is available on the relationships between the bracket material, plaque accumulation and the micro-organisms. The purpose of this in vivo study was to determine the presence of *S. mutans* and *S. Sobrinus* on orthodontic brackets made from four different materials and the possible correlation between the prevalence of *S. mutans* and *S. sobrinus* and the VPI and GBI.

Materials and methods

Forty subjects (Mean age: 21.3 years; Range: 17.6–25.9 years) with Class I malocclusions and less than 2.5 mm crowding were selected for this study. The subjects were matched for caries experience and the first premolars were extracted as part of the orthodontic treatment plan. Stainless steel brackets (.022 x .028 inch MBT, Victory, 3M Unitek, Monrovia, CA, USA) were bonded to the upper and lower teeth, except the second premolars, using Transbond XT composite resin (3M Unitek, Monrovia, CA, USA). Using a split mouth design[10] and a randomisation table, either a stainless steel bracket (Victory, 3M, Unitek, Monrovia, CA, USA), a gold bracket (Magnum Gold, Orthodontic Design and Production Incorporated, Vista, CA, USA), a ceramic bracket (InVu Ceramic, TP Orthodontics Inc, La Porte, IN, USA) or a plastic bracket (Brilliant, Forestadent, Pforzheim, Germany) were bonded to the second premolar in each quadrant. The design ensured that the brackets made of the different materials were equally distributed to the quadrants since the location of the bracket was considered a possible factor in bacterial adhesion.

After bonding the study brackets, 0.016 inch NiTi archwires (3M Unitek, Monrovia, CA, USA) were ligated with elastomeric modules. The same post-bonding instructions were given to all subjects and no antimicrobial mouthwashes were prescribed. The patients were recalled after 30 days for review. Subjects with debonded brackets within this period were eliminated from the study. The VPI and GBI were used to assess the amounts of plaque and gingival bleeding around the second premolars. Four gingival areas of the tooth (facial, mesial, distal and lingual) were assessed by running a periodontal probe along the soft tissue wall of the gingival crevice for presence of plaque and gingival bleeding and scored from 0 to 3. A score of 0 meant normal gingiva and a score of 3 meant severe inflammation.

Debonding procedure

The second premolar brackets were carefully removed using debonding pliers and placed in eppendorf jars containing 100 µl of sterile normal saline. Chromosomal DNA was immediately extracted from the adhering bacteria. The 100 µl sample was diluted with an equal volume of 0.1M HCl-Tris buffer at a pH of 8.0. Following a previous protocol,[11] the mixture was incubated at 100 °C for 60 minutes, centrifuged at 10,000 g for 5 minutes and the supernatant stored at 4 °C for later PCR assay.

Known specific primers that amplify the dextranase gene of *S. mutans* and *S. sobrinus* were selected.[6,12] For *S. mutans* the upper primer - 5'-TAT GCT GCT ATT GGA GGT TC-3' is complementary to the sequence 973 to 992 and the lower primer - 5'-AAG GTT GAG CAA TTG AAT CG-3' is complementary to the sequence 2225 to 2244. The size of the expected PCR product was 1215 base pairs (bp). For *S. sobrinus* the upper primer 5'-TAC TAT CTT TCC CTA GCA TG-3' is complementary to the sequence 134 to 153, and the lower primer 5'-GGT ATT CGG TTT GAC TGC-3' is complementary to the sequence 1743 to 1726.The size of the expected PCR product was 1610 bp. All primers were commercially synthesised (Synergy scientific services, Chennai, India). Taq polymerase buffer 5 µl, dNTP mix 2 µl, primers 2 µl, DNA template 20 µl, Taq polymerase

Table I. Prevalence of *S. mutans*, *S. sobrinus* and cariogenic streptococci on premolar brackets.

Groups	Brackets (Number)	*S. mutans* Mean ± SD	*S. sobrinus* Mean ± SD	Cariogenic streptococci Mean ± SD
Stainless Steel (SS)	40	0.38 ± 0.49	0.28 ± 0.45	0.65 ± 0.89
Gold	40	0.25 ± 0.44	0.20 ± 0.41	0.45 ± 0.82
Ceramic	40	0.88 ± 0.34	0.75 ± 0.44	1.63 ± 0.71
Plastic	40	0.75 ± 0.44	0.58 ± 0.50	1.33 ± 0.86
Total	160	0.56 ± 0.50	0.45 ± 0.50	1.01 ± 0.95

Table II. Multiple comparisons with Post Hoc tests.

(I) Group	(J) Group	*S. mutans* Mean difference (I vs J)	*p*	*S. sobrinus* Mean difference (I vs J)	*p*	Cariogenic streptococci Mean difference (I vs J)	*p*
SS	Gold	0.13	0.56	0.75	0.88	0.20	0.70
	Ceramic	-0.50*	**0.00**	-0.48	**0.00**	-0.98*	**0.00**
	Plastic	-0.38*	**0.00**	-0.30	0.02	-0.68*	**0.00**
Gold	SS	0.13	0.56	0.75	0.88	-0.20	0.70
	Ceramic	-0.63*	**0.00**	-0.55	**0.00**	-1.18*	**0.00**
	Plastic	-0.50*	**0.00**	-0.38	**0.00**	-0.88*	**0.00**
Ceramic	SS	0.50*	**0.00**	0.48	**0.00**	0.98*	**0.00**
	Gold	0.63*	**0.00**	0.55	**0.00**	1.18*	**0.00**
	Plastic	0.13	0.56	0.18	0.31	0.30	0.36
Plastic	SS	0.38*	**0.00**	0.30	0.02	0.68*	**0.00**
	Gold	0.50*	**0.00**	0.38	**0.00**	0.88*	**0.00**
	Ceramic	-0.13	0.56	-0.18	0.31	-0.30	0.36

* $p < 0.05$
Significant values in bold

enzyme 0.5 µl and double distilled water 20.5 µl were added to the PCR tubes to make a total of 50 µl. The PCR tubes were then subjected to thermocycling and the sample was subsequently presented for agarose gel electrophoresis. The generated electrophoretic bands were examined with a UV transilluminator (Figure 1).

Statistical analysis

The data were analysed with SPSS version 15 (SPSS Incorporated, Chicago, IL, USA). One-way ANOVA and Scheffé post-hoc tests were used to compare the prevalence of each micro-organism adhering to the brackets and their relationships with the oral hygiene indices. A value of $p < .05$ was considered significant.

Results

A positive PCR response indicated that the number of streptococci adhering to the brackets was over 1000 cells. The gold brackets had the least number of bacteria and the ceramic brackets had more than twice the number of bacteria as the gold brackets (Table I). The prevalence of the bacteria increased from the gold brackets (Mean: 0.25 ± 0.44), to the stainless steel brackets (Mean: 0.38 ± 0.49), to the plastic brackets (Mean: 0.75 ± 0.44) and finally, to the ceramic brackets (Mean: 0.88 ± 0.34). There was no statistically significant difference between either the gold and stainless brackets or between the plastic and ceramic brackets (Table II). Similar results were found for the prevalence of *S. sobrinus* alone and

Table III. Relationship between prevalence of ± *S. mutans* and *S. sobrinus* and Visible Plaque Index (VPI) and Gingival Bleeding Index (GBI) on various premolar brackets with One-way ANOVA.

S. mutans	*S. sobrinus*	VPI		GBI	
		Brackets (Number)	Mean score ± SD	Brackets (Number)	Mean score ± SD
N	N	37	0.32 ± 0.48	37	0.19 ± 0.40
N	P	23	0.43 ± 0.51	23	0.39 ± 0.50
P	N	33	0.52 ± 0.51	33	0.48 ± 0.51
P	P	67	0.93 ± 0.27	67	0.88 ± 0.32
Total		160	0.63 ± 0.48	160	0.59 ± 0.49

N = negative, P = positive
$p < .05$

S. mutans and *S. sobrinus* together on the different bracket materials.

For all brackets, the VPI and GBI correlated well and were equally accurate in predicting the prevalence of *S. mutans* and *S. sobrinus* (Table III). For 37 of the brackets, *S. mutans* and *S. sobrinus* were not sensitive to PCR and therefore not detected. The associated VPI and GBI yielded smaller mean values (VPI: 0 .32 ± 0.48; GBI: 0.19 ± 0.40). The highest prevalence was found on 67 brackets in which both *S. mutans* and *S. sobrinus* were sensitive to PCR (VPI: 0.93 ± 0.27; GBI: 0.88 ± 0.33). ANOVA showed a significant relationship between the prevalence of *S. mutans* and *S. sobrinus* and the oral hygiene indices.

Discussion

Enamel demineralisation is caused by organic acids produced mainly by *S. mutans* and its variant, *S. sobrinus*.[13,14] Patients undergoing fixed appliance orthodontic treatment are at greater risk of enamel demineralisation. The aim of the present study was to investigate the presence and prevalence of *S. mutans* and *S. sobrinus* on different bracket materials using the polymerase chain reaction (PCR) technique and to correlate the prevalence of these streptococci with the Visible Plaque Index (VPI) and Gingival Bleeding Index (GBI). The results of the present study show that microbial adhesion was greater around aesthetic brackets. Also, the prevalence of *S. mutans* and *S. sobrinus* was significantly associated with oral hygiene indices in the premolar regions. A lack of oral hygiene was strongly associated with the accumulation of cariogenic streptococci in this area and therefore may be considered as an indicator of bacterial population.

Gwinnett et al.,[15] has shown that extensive plaque accumulation is associated with bonded orthodontic brackets and Mitchell[16] has documented that fixed appliances with orthodontic brackets caused specific changes in the oral environment, such as decreased pH and increased plaque accumulation. The bacterial adhesion to orthodontic brackets may be a primary step leading to plaque accumulation and, because the adhering bacteria continue to grow on the tooth surfaces near the brackets, enamel demineralization around brackets is likely.[7] Since the initial affinity of bacteria to solid surfaces is mostly due to electrostatic and hydrophobic interactions, the bracket material could play a role in the level of bacterial adhesion and plaque accumulation that leads to an increased risk of caries.

The finding that there were fewer *S. mutans* and *S. sobrinus* on the metal brackets and more on the aesthetic brackets agrees with the findings of Ahn et al.[7] However, other reports have indicated that there were no obvious differences in the adhesion of *S. mutans* to stainless steel, plastic and ceramic brackets.[17–19] This may be explained by the differences in the detection methods.

The adhesion of micro-organisms to orthodontic brackets is a complex and poorly understood process. Microbial adhesion has been attributed to the surface energy of the substrata (i.e. the bracket material), to an adsorbed salivary protein layer or to surface roughness of the bracket.[20–23] Van Dijk et al.[20] showed greater bacterial adherence to surfaces with high

surface energy and Eliades et al.[21] reported that stainless steel, because of a high critical surface tension, has increased attachment potential. However, these observations might not be directly applicable as Weerkamp et al.[22] reported that the adhesion of oral micro-organisms to bracket surfaces was governed by the properties of an adsorbed protein layer. Although Eliades et al.[21] and Bauer et al.[23] showed that plastic materials have lower surface-free energy than metal bracket materials, the variation can be explained by the surface energy characteristics of plastic brackets which are strengthened by the addition of filler. The combination of plastic and filler may produce rough surfaces which increase the adherence and retention of cariogenic streptococci.

The results of the present study have shown a significant relationship between the prevalence of streptococcal organisms and the oral hygiene indices. It follows that the patient's oral hygiene status is associated with cariogenic streptococci and therefore considered as an indicator of this bacterial population. Nyvad et al.[24] reported that the proportion of *S. mutans* is significantly lower and comprised only 0.5 per cent of dental plaque after 24 hours. Although the population of streptococci could be small, the high sensitivity of the PCR technique and its ability to detect as few as 1000 cells, offers confidence that the oral hygiene indices provided reliable measures of cariogenic streptococci presence.

The duration of the present study was 30 days even though it is recognised that plaque formation can occur within 24 hours. It was expected that a 30-day period would be sufficient to assess the oral hygiene routine of the patients. The significant relationship between the oral hygiene indices and bacterial prevalence suggests that the clinician should critically evaluate the patient's oral hygiene practises to prevent white spot formation during and prior to treatment.

The results of the present study indicate that aesthetic brackets are more likely to attract cariogenic bacteria and therefore increase the risk of enamel decalcification. Future studies with bracket raw materials are required to identify the main component in the bracket that causes the attraction. Furthermore, the patient's diet, oral hygiene including types of toothbrushes, toothpastes and mouthwashes, the type of adhesive and even fixed appliance components could affect the adhesion and prevalence of cariogenic bacteria. It is suggested the use of aesthetic brackets requires the consideration of additional oral hygiene aids that reduce plaque accumulation.

Conclusion

The results of this study suggest that oral hygiene is of greater importance when aesthetic brackets are used for fixed appliance treatment.

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