Salivary *Streptococcus mutans* levels in patients with conventional and self-ligating brackets

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SUMMARY The objective of this study was to investigate the effect of bracket type (conventional and selfligating) on the levels of *Streptococcus mutans* and total bacterial counts in whole saliva of orthodontic patients. Thirty-two male and female patients were selected using the following inclusion criteria: adolescents (mean age 13.6 years, range 11–17 years), fixed appliances in both arches, non-smoker, and no reported oral habits. Demographic and oral hygiene characteristics were determined for each subject. The patients were subdivided into two groups with random allocation of bracket type (conventional or selfligating). An initial saliva sample was obtained before the initiation of treatment (T1) and a second sample 2–3 months following appliance bonding (T2). Salivary *S. mutans* and total bacteria were enumerated and analysed after growth in culture. The demographic and clinical characteristics of the samples were analysed with a *t*- or chi-square test, where applicable, to assess the random allocation of bracket group to participants. The results of *S. mutans* and total facultative bacterial counts were log transformed and statistically analysed with analysis of covariance with bracket (conventional versus self-ligating) as the categorical variable and initial total bacterial counts or initial *S. mutans* levels serving as the covariate.

No difference was found in the demographics and oral hygiene indices between the two groups, verifying the random assignment of brackets to the population sample. The levels of *S. mutans* in whole saliva of orthodontically treated patients do not seem to be significantly different between conventional and self-ligating brackets. The pre-treatment levels of *S. mutans* are significant predictors of the levels of *S. mutans* after placement of orthodontic appliances, while this was not the case for total bacterial counts.

Introduction

Although self-ligation in orthodontics was described several decades ago, the first commercially viable system, the Speed bracket, was not introduced until the early 1980s. The late 1990s marked a significant increase in the popularity of self-ligation with almost all major orthodontic companies listing them among their products (Harradine, 2003).

One of the proposed favourable aspects of self-ligating brackets is the elimination of elastomeric or stainless steel ligatures. It has been proposed that this feature brings two basic advantages: the eradication of cross-contamination, which may accidentally be involved in the process of ligature change, and the claimed improvement in the oral hygiene of patients. The latter has been attributed to the fact that the patient is given the opportunity to clean surfaces of reduced complexity and with less retentive sites for microbial colonization (Øgaard *et al.*, 1988).

The oral cavity is a rich ecosystem with a plethora of microorganisms. While both periodontal disease and caries are considered multifactorial diseases, plaque bacteria are the major factor in their onset and progression. However, there are situations which comprise what has been termed 'ecological stress', referring to the shift of the microbiological balance, creating conditions conducive to the growth, and appearance of cariogenic and/or periodontopathic bacteria (Marsh, 2003).

The different components of the fixed orthodontic system may contribute to a shift in the balance of the oral ecology. A large amount of research has dealt with the intimate contact of orthodontic materials with the tooth and periodontal tissue. The presence of brackets and ligatures has been shown to be related to an increase in gingival inflammation and increased risk of decalcification. This demineralization of the tooth surfaces results in the appearance of white spots or even carious lesions (Gorelick *et al.*, 1982; Øgaard *et al.*, 1988; Fournier *et al.*, 1998; Naranjo *et al.*, 2006).

Oral microbiota attachment in orthodontic patients has been mainly associated with increased risk of *Streptococcus mutans* and lactobacilli colonization, among other species, thus initiating a series of events, which may lead to the development of pathology of the hard tissues such as decalcification and, in specific cases, caries development (Gorelick *et al.*, 1982; Øgaard *et al.*, 1988; Fournier *et al.*, 1998). Moreover, the accumulation of plaque and the resultant alteration of the local microbial milieu may expose the tissues to the risk of developing periodontal inflammation, with notable changes in the biota (Naranjo *et al.*, 2006).

Even though the aforementioned effects have been studied extensively, there is a lack of substantiation of the hypothesis of decreased plaque retention related to the use of self-ligating brackets. The hypothesis investigated in the present research was that bracket ligation mode has an effect on the microbiological profile of the patients' oral environment. Therefore, the objectives of this study were to investigate the effect of bracket type (conventional or self-ligating) on salivary *S. mutans* counts and total bacterial count levels in patients undergoing treatment with conventional and self-ligating appliances.

Subjects and methods

The individuals participating in the study group were selected from a larger pool of patients from the practice of one author (NP), using the following inclusion criteria: adolescents treated with fixed appliances, no reported oral habits detrimental to health, including smoking, absence of restorations, and/or missing teeth due to dental caries. The patients who fulfilled these criteria were randomly assigned to one of the treatment groups. The orthodontist did not know the bracket group assignment at the time of the first saliva collection. Consent for saliva collection was obtained from all patients.

The 32 male and female patients were divided into two equal groups based on bracket type used, i.e. conventional (Microarch, GAC International, Central Islip, New York, USA), ligated with conventional (no fluoride-releasing) elastomeric modules, or self-ligating (In-Ovation-R, GAC International). From previous studies (Forsberg *et al.*, 1991; Attin *et al.*, 2005), it is inferred that a mean colony-forming unit (CFU) difference of approximately one log [standard deviation (SD) = approximately 1] will result in a clinically significant increase in *S. mutans* counts. Therefore, the sample size of 16 patients per group, at α =0.05, yields a statistical power very close to 0.80 for this study.

Whole stimulated saliva was collected from each patient at two time points before bonding and initiation of orthodontic therapy (baseline at T1) and after at a period of 2–3 months (T2) after full bonding had taken place. At both time points, each subject was asked to collect saliva in the mouth and to expectorate into a chilled empty Petri dish approximately 3 ml of saliva. The subjects had refrained from eating or drinking beverages for at least 1 hour before saliva collection. Collection of saliva samples was performed before any oral examination or manipulation so as not to disrupt the oral microbiota.

Standard oral hygiene instructions were provided at the beginning of treatment using a model, with specific attention to the orthodontic appliances. Prior to all examinations, no brushing or other hygiene measures were carried out. For each participant, the following clinical variables were assessed: the simplified plaque index (S-PII), where the percentage of surfaces with plaque is recorded (taking into consideration all surfaces per tooth for all erupted teeth), and the decayed, missing, and filled teeth (DMFT) index for the prevalence of caries. Both indices were recorded after each saliva sample collection at each visit without the use of a plaque disclosing agent.

Serial 10-fold dilutions of the saliva samples were inoculated on a non-selective medium and on a selective growth medium for *S. mutans*. Aliquots of 0.1 ml of the dilutions were inoculated onto non-selective blood agar plates supplemented with 7 per cent sterile blood for the evaluation of the composition of the predominant cultivatable microbiota of the saliva. The blood agar plates were incubated at 37° C for 3 days in a CO₂ atmosphere, following which the total number of CFU was counted.

The selective medium used was Mitis Salivarius agar supplemented with sucrose (20 per cent w/v), bacitracin, and tellurite solution. The plates were incubated for up to 7 days in a CO₂ atmosphere at 37°C. The above process was repeated 2–3 months after the orthodontic appliances were bonded. The presumptive characterization and identification of *S. mutans* was based upon colony morphology, Gram stain, and catalase activity. From these, representative colonies were subcultured and biochemical tests performed for definitive species identification. All laboratory procedures were carried out without the personnel knowing the allocation of saliva samples to bracket groups. Additionally, in an effort to minimize variation, the *S. mutans* counts were also recorded as a proportion of total bacterial counts (*S. mutans* counts/total bacterial counts) at T1 and T2.

Demographic and clinical characteristics of the sample were investigated with conventional descriptive statistics. Differences of means (gender, age, S-PII, DMFT, and days between T1 and T2 sample) were determined with a *t*-test, whereas differences in proportion (males–females proportion between the two groups) were studied with the chi-square test.

Comparisons of the total cultivatable counts (total number of CFU) and total CFU of *S. mutans* per millilitre of saliva between the two bracket groups were performed independently; they were log transformed and statistically analysed with analysis of covariance (ANCOVA) with bracket (conventional versus self-ligating) as the categorical variable and initial total bacterial counts or initial *S. mutans* levels serving as the continuous covariate. Data were further analysed with simple linear regression analysis to evaluate the initial levels of facultative total bacteria counts and *S. mutans* as predictors of the levels of these at the second time interval. Data analysis was conducted with the statistical package, Minitab 14.20 (State College, Pennsylvania, USA), at the 0.05 level of significance.

Results

Table 1 shows the distribution of demographic, oral hygiene variables, and the average number of days between the T1 and T2 saliva samples for the two groups. The distribution of gender, age, and treatment duration prior to the T2 saliva sample did not show any difference between the two bracket groups verifying the random selection of these variables across the two populations. Additionally, the oral hygiene status as determined by the S-PII and DMFT indices for the prevalence of caries are shown.

The mean logs and SDs for total bacterial and *S. mutans* counts at T1 and T2 sample collection for the two bracket groups are shown in Table 2. Statistical analysis showed no difference with respect to total bacterial and *S. mutans* counts between the two bracket groups at either T1 or T2.

ANCOVA results for total salivary microbial counts for the conventional and self-ligating bracket groups are shown in Table 3. No statistically significant difference was

 Table 1
 Demographic and clinical characteristics of the subjects included in the study.

Variable—bracket group	Mean	SD	P level	
S-PII—conventional (initial, %)	48.1	13.2	NS	
S-PII—self-ligating (initial, %)	47.3	9.8		
S-PII—conventional (2–3 months)	46.1	8.4	NS	
S-PII—self-ligating (2–3 months)	46.5	6.6		
DMFT—conventional	1.4	0.4	NS	
DMFT—self-ligating	1.8	0.4		
Gender ratio—conventional Gender ratio—self-ligating	0.5 0.5		NS	
Age (years)—conventional	13.38	1.5	NS	
Age (years)—self-ligating	13.81	1.5		
Days from first sample—conventional Days from first sample—self-ligating	84.5 89.9	5 5	NS	

NS, non-significant; S-PII, simplified plaque index for percentage of surfaces with plaque considering all surfaces per tooth; DMFT, decayed, missing, and filled teeth, total indicating the sum of DMF.

Table 2 Mean and standard deviation (SD) of log-total bacteria and *Streptococcus mutans* counts per millilitre saliva before the start of treatment (T1) and following appliance bonding (T2) for the bracket groups.

Variable	Bracket type	Mean	SD	P level
Log-total count T1	Conventional	6.78	1.79	NS
0	Self-ligating	7.24	2.09	
Log-total count T2	Conventional	7.76	1.32	NS
0	Self-ligating	7.24	2.09	
Log-S. mutans T1	Conventional	4.53	1.02	NS
0	Self-ligating	3.98	0.87	
Log-S. mutans T2	Conventional	4.66	1.45	NS
0	Self-ligating	4.48	0.83	

NS, non-significant.

detected between the bracket groups. Additionally, total bacterial counts at T1 were not found to be significant predictors of the total bacterial counts at T2.

The corresponding ANCOVA for the S. mutans levels are illustrated in Table 4; again the variable 'bracket' had no effect as depicted in Table 3, where no difference between the bracket groups was identified. However, the T1 levels of S. mutans in saliva (the covariate) were shown to be significant predictors of the levels of S. mutans at T2. This was also confirmed by simple linear regression (r=0.474, P=0.022). The low values for the adjusted r^2 signify the low fit of the model with regard to the variables used, which means that the variable bracket type and initial S. mutans counts (T1) in combination are not good predictors of S. mutans counts at T2. However, initial S. mutans counts on their own are significant predictors (P=0.033) of the final S. mutans counts (Draper and Smith, 1988; Kim and Dailey, 2008). Some of the individuals had no detectable S. mutans counts and the zero values were not included in the model because they would have produced misleading results by lowering the means and thus the S. mutans counts would have been under-represented.

Table 5 shows the mean logs and SDs for the ratio of *S. mutans* counts to total bacterial counts. Again, statistical analysis showed no difference in total *S. mutans*/total bacterial count ratios between the two bracket groups at T1 and T2, confirming the results of the previous analyses.

Discussion

The initial affinity of bacteria to solid surfaces is mostly due to electrostatic and hydrophobic interactions, while surfaces with high surface-free energy more easily attract bacteria such as S. mutans (Van Dijk et al., 1987). A recent in vitro study (Papaioannou et al., 2007) assessed the adhesion of a clinical strain of S. mutans on brackets of different composition, with a saliva coating as well as uncoated. The effect of Streptococcus sanguinis was also examined by allowing these bacteria to adhere before the adhesion of S. mutans. It was clear from the results that there were no significant differences in the adherence of S. mutans to the three different types of brackets. For uncoated brackets, it is expected that only surface characteristics would determine adhesion of bacteria, suggesting that bacteria with high surface-free energy such as S. mutans (Weerkamp et al., 1985) should prefer surfaces with high surface-free energy materials such as stainless steel brackets. However, this has not been confirmed (Fournier et al., 1998; Ahn et al., 2005; Papaioannou et al., 2007), and therefore, variations in surface-free energy in conjunction with other surface properties of bracket raw materials (Eliades et al., 1995) may not produce a measurable effect on adhesion.

Even though the attachment of plaque on bracket surfaces constitutes a direct assessment of the effect of bracket on microbial colonization, this approach presents major

Source	df	Sequential sum of squares	Adjusted sum of squares	Adjusted mean of squares	F	P level	
Log-total counts	1	0.63	0.73	0.734	0.56	0.458	NS*
Bracket	1	0.34	0.34	0.335	0.26	0.615	NS†
Error	29	37.67	37.67	1.299			
Total	31	38.63					
S=1.13970	$R^2 = 2.49\%$		$R^2(adjusted) = 0.00\%$				

Table 3 Analysis of covariance for the salivary total microbial counts per millilitre of saliva of the subjects included in the study.

*NS, non-significant. The initial (T1) log-total bacterial counts are not a significant predictor of the log-total bacterial counts at T2 (after bonding). †NS, non-significant difference on the log-total bacterial counts between the two bracket groups, adjusted for initial log-total bacterial counts.

Table 4 Analysis of covariance for the salivary Streptococcus mutans counts per millilitre saliva of the subjects included in the study.

Source	df	Seq SS	Adjusted sum of squares	Adjusted mean of squares	F	P level	
Log-S. mutans	1	6.09	5.47	5.47	5.25	0.033*	
Bracket	1	0.16	0.17	0.17	0.16	0.694	NS†
Error	20	20.85	20.84	1.04			
Total	22	27.10					
S=1.02095	$R^2 = 23.09\%$		R^2 (adjusted)=15.39%				

*Significant. The initial (T1) log-S. mutans counts is a significant predictor of the log-S. mutans counts at T2.

*NS, non-significant difference on the log-S. mutans counts between the two bracket groups, adjusted for initial log-S. mutans counts.

Table 5 Means and standard deviations (SDs) of the ratio of log-*Streptococcus mutans* to log of total bacterial counts (TC) per millilitre saliva at baseline (T1) and following bonding (T2) for the bracket groups.

Variable	Bracket type	Mean	SD	P level
Log-S. mutans/log-TC@T1	Conventional Salf ligating	0.65	0.18	NS
Log-S. mutans T2/log@TC	Conventional Self-ligating	0.62 0.59 0.61	0.29 0.17 0.11	NS

NS, non-significant.

technical and methodological difficulties. First, microbial accumulation may follow different rates at various intraoral sites as a result of the proximity of the bracket to the gingival sulcus, the surface area of the labial enamel surface relative to the bracket, and the position relative to the mandibular glands. At low flow rates or under static conditions, the grooves of rough surfaces may act as stagnation points, thereby promoting biofilm maturation (Karino *et al.*, 1987). Also, elastomeric ligatures are frequently changed and therefore, the time elapsed after their renewal may have an effect on bacterial attachment since the substrate for colonization is eliminated and a new cycle of colony formation is initiated on new material.

On the other hand, direct assessment of microbes *in situ* and onto specific areas may not at present be feasible. It

follows that for a general assessment of microbial colonization on tooth and bracket surfaces, salivary sampling may be selected based on the assumption that salivary levels of microbia may represent the variation of those attached to bonded teeth. The available literature supports such a correlation between the presence of *S. mutans* in saliva and plaque (Mundorff *et al.*, 1990; Sullivan *et al.*, 1996). High counts in the saliva usually correlate to more than 10^3 mean squares in the plaque. In the present study, the levels were around 10^4 before and after bonding in both groups. This is most probably due to the low levels of caries experience of both groups.

Other factors in the oral environment may further decrease any possible variations due to the different surface characteristics. One such 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 may be influenced to a large extent by interactions between salivary components in the pellicle and the properties of the different microorganisms, in addition to the adherent patterns of bacteria on the different types of orthodontic brackets. The presence of an early salivary pellicle has been found to reduce the number of adhering bacterial cells of *S. mutans* (Fournier *et al.*, 1998; Papaioannou *et al.*, 2007).

An interesting observation relates to the interaction between different bacterial species in adhesion to a surface. Specifically, *S. sanguinis*, one of the initial colonizers of the oral cavity, has been found to further reduce the number of adhering *S. mutans* regardless of the type of surface. *Streptococcus mutans* and *S. sanguinis* would seem to have an antagonistic relationship and early colonization by the latter may have a significant effect on concentrations of *S. mutans* (Quirynen and Bollen, 1995). A delayed colonization by *S. mutans* may lead to less caries or caries susceptibility.

The morphology and design of orthodontic brackets, as well as the ligation mode may play a role by providing an increased number of retention sites as well as protection from plaque-removing shear forces arising from masticatory loads and fluid flow, thus facilitating dental plaque accumulation and maturation, and consequently increased levels of S. mutans in saliva. One of the proposed favourable aspects of self-ligating brackets is associated with the elimination of elastomeric or stainless steel ligatures. Teeth ligated with elastomeric ligatures, as in the present study, have been found to harbour in the area of the brackets, higher numbers of bacteria than those where steel wire is utilized (Caufield et al., 2000). The type of bacterial morphotypes, as detected in a scanning electron microscopy study, was not, however, found to differ between the two ligation methods (Sukontapatipark et al., 2001). Türkkahraman et al. (2005), employing a split-mouth protocol, examined the effect of the two ligation modes (elastomeric rings and ligature wires) on the accumulation of specific cariogenic species (S. mutans and lactobacilli) as well as the periodontal status, before therapy and at 1 and 5 weeks after treatment initiation. Slightly higher total counts of bacteria around the elastomeric rings were found that, however, did not reach statistical significance, even though, in all 21 patients, significant increases of bacteria counts were recorded. Finally, elastomeric rings appeared more conducive to gingival bleeding, perhaps due to their slightly higher affinity to plaque. For this reason, those authors suggested that the use of elastic ligatures should be avoided in patients with inadequate oral hygiene.

The hypothesis of the present study that self-ligating bracket should have a beneficial effect due to the absence of ligatures, is not confirmed. However, as shown (Table 2), although the oral concentrations of *S. mutans* were found to be slightly lower in patients with self-ligating compared with conventional brackets, this difference did not reach significance. There seemed to be a small effect on the total bacterial load of the saliva, with the patients bonded with the self-ligating brackets showing a smaller increase of load at T2 compared with the conventional group, but again no significant effect was detected.

Based on the present findings, total facultative bacterial and *S. mutans* counts in saliva do not seem to be significantly different between the bracket groups tested. Locally though, i.e. in the tissues adjacent to brackets and peripheral marginal adhesive resin, the situation may be totally different and there may be an effect. For the self-ligating brackets, the data are extremely poor. Only one study is presently available and that focuses on periodontal factors and associated bacteria. In that study (van Gastel *et al.*, 2007), an important local effect of bracket type was found. Indeed, at the area around the brackets, there were significant alterations in both periodontal and microbiological parameters, with the self-ligating Speed brackets showing poorer scores.

The results of the present study suggest that bracket design parameters may not have a significant effect on bacterial colonization on orthodontic appliances. This could be attributed to the implementation of an oral hygiene programme, which is taught at the early stages of orthodontic treatment, and the potential minute role of bracket design to offer sites for microbial adherence when such oral hygiene is exercised. However, the initial concentrations of *S. mutans* did exert a significant effect upon the counts of this bacterium over time. This may be an important factor to take into consideration when determining the risk a specific patient may be exposed to, thus necessitating a more individualized preventive programme.

Other approaches to alter the relationship between ligature elastomers and dental plaque accumulation have often been sought. The most common includes the use of fluoride-releasing elastomers (Wilson and Gregory, 1995; Benson et al., 2004). Stannous fluoride is the fluoride of choice when the focus is placed on bacteria due to the antibacterial properties it possesses (Camosci and Tinanoff, 1984). A significant decrease in salivary levels was found when fluoride-releasing elastomers were placed in a group of orthodontic patients; however, there was no significant effect after 2 or more weeks of retention of elastomers (Wilson and Gregory, 1995). In a more recent split-mouth crossover study (Benson et al., 2004), the bacteria that the elastomeric rings retained were examined after 6 weeks of intraoral use. No significant differences in culture growth for streptococcal and anaerobic bacteria were found.

Conclusion

Total bacterial counts in whole saliva did not differ significantly among patients with conventional and self-ligating brackets. Additionally, bracket type (conventional versus self-ligating) does not seem to significantly alter the levels of *S. mutans* in whole saliva.

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