

Effect of the bracket types on microbial colonization and periodontal status

Fatma Deniz Uzuner^a; Emine Kaygısız^b; Zeynep Turgut Çankaya^c

ABSTRACT

Objective: To evaluate the effect of different bracket types on the levels of *Streptococcus mutans* (SM) and *Lactobacillus* (LB) in saliva, in plaque, and on the periodontal condition.

Materials and Methods: Forty patients aged 14 to 16 years, who had Angle Class I malocclusion with minimal crowding, were nonsmokers, were without systematic disease, and did not use antibiotics or oral mouth rinses during the 3-month period before the study were randomly selected. The patients were subdivided into two groups with random allocation of bracket type: conventional brackets (CB; Avex Mx, OPAL orth.) with steel wire ligature or self-ligating brackets (SLB; F1000, Leone S.p.A.). Microbial and periodontal records were obtained before bonding (T₁) and 1 month after bonding (T₂). Microbial samples were collected from the stimulated saliva and the plaque from the labial surfaces of the upper and lower lateral incisors. To estimate the number of colony-forming units of SM and LB, Dentocult SM and LB kits were used. The plaque index (PI), gingival index (GI), and pocket depth (PD) values were recorded to evaluate the periodontal condition. Paired *t*-test and Mann-Whitney *U*-test were used to compare the groups statistically.

Results: No significant differences occurred in SM or LB colonization between the groups. In the SLB group, PI, GI, and PD values increased significantly ($P < .05$). A greater increase was found in PD value in the SLB group (0.98 mm) compared with the CB group (0.04 mm; $P < .05$).

Conclusions: The F1000 SLB do not have an advantage over Avex Mx CB with respect to periodontal status and colonization of SM and LB. (*Angle Orthod.* 2014;84:1062–1067.)

KEY WORDS: Microbial colonization; Periodontal status; Self-ligating brackets; Conventional brackets; Dentocult kits

INTRODUCTION

Fixed orthodontic appliances with the presence of brackets and ligatures create new retention areas for microbial dental plaque that is reported to be the most important etiologic factor in periodontal inflammation and decalcification of enamel.^{1–4} In conjunction with poor oral hygiene, the number and percentage of oral microorganisms are increasing during fixed orthodontic treatment.^{5,6} Among other species, *Streptococcus*

mutans (SM) and *Lactobacillus* (LB) bacteria, which have been identified with high concentration in oral flora and have higher adhesion capacity on brackets, play important roles in the initiation and progression of dental caries.^{7–9}

The effects of bracket materials, designs and different ligations on the levels of cariogenic bacteria and periodontal destruction have been evaluated by several studies.^{4,10–15} Among bracket ligation types, self-ligating brackets (SLB) were expected to have better values for periodontal status because of the lack of ligature materials and fewer retentive sites.⁴ On the contrary, others^{16,17} have mentioned that this theoretical advantage may be eliminated in reality, where SLB consist of opening and closing mechanisms that may provide additional plaque retention sites.

There are few studies in the literature with diverse results comparing SLB with elastomeric ligated conventional brackets (CB).^{18–23} Some researchers have reported that elastomeric ligated CB cause more plaque accumulation and periodontal inflammation than SLB,^{6,18,19} whereas others reported no significant difference.²⁰ On the contrary, higher bacterial colonization

^a Specialist, Department of Orthodontics, Gazi University Faculty of Dentistry, Ankara, Turkey.

^b Lecturer, Department of Orthodontics, Gazi University Faculty of Dentistry, Ankara, Turkey.

^c Research Assistant, Department of Periodontology, Gazi University Faculty of Dentistry, Ankara, Turkey.

Corresponding author: Dr Emine Kaygısız, Department of Orthodontics, Gazi University Faculty of Dentistry, Biskek Cd. (8.Cd.) 82.Sk. No:4, 06510 Emek, Ankara, Turkey (e-mail: dt.emineulug@mynet.com)

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and poorer periodontal health with SLB was also reported.²¹

On the other hand, there is no clinical study to our knowledge evaluating the effects of SLB and CB with wire ligation on oral microbiota and periodontal status. Thus, this prospective study was performed to investigate the effect of fixed orthodontic treatment with SLB and CB with steel ligatures on the colonization of SM and LB in saliva, in plaque, and on the status of periodontal health. The null hypothesis assumed that bracket type has no effect on these parameters.

MATERIALS AND METHODS

Forty patients (29 female, 11 male) between the ages of 14 and 16 years were randomly selected from among the patients scheduled for orthodontic treatment in the orthodontic department of the university according to the following inclusion criteria: permanent dentition, Angle Class I malocclusion with minimal crowding, nonsmoker, and without periodontal and systematic disease. Patients who had used antibiotics and oral mouth rinses during the 3-month period before the study were excluded. Patients used standardized fluoridated toothpaste and toothbrush and were asked not to use other oral hygiene supplements during the study. Oral hygiene instructions were given to all patients 1 week before the beginning of treatment.

This study was approved by the Ethics Committee of the University. All patients/parents were informed, and their consent was given prior to entering the study.

It is inferred that a mean colony-forming unit (CFU) difference of approximately one score (standard deviation = approximately 1) will result in a clinically significant increase in SM and LB counts. Although a sample size of 16 patients per group, at $\alpha = .05$, yields a statistical power very close to .80 for this study, the sample size was increased to 20 patients per group.

Patients were subdivided into two groups with random allocation of bracket type as follows: group 1: conventional (CB; Avex Mx, 0.022-inch, OPAL orth, South Jordan, Utah; $n = 20$; Figure 1) and group 2: SLB (F1000, 0.022-inch, Leone SpA, Sesto Fiorentino, Firenze, Italy; $n = 20$; Figure 2). CBs were ligated with 0.010-inch stainless-steel wire.

Microbial and periodontal records were obtained before upper and lower arch bonding (T_1) and 1 month after bonding (T_2), which is the time for the appointment to change the first arch wires. All microbial and periodontal records were collected by a single calibrated periodontist; a complete periodontal examination was performed by means of a Williams probe. Periodontal examination included the plaque index



Figure 1. Avex Mx conventional brackets.

(PI), gingival index (GI), and probing pocket depth (PD) measurements at four sites (mesial, midbuccal, distal, and palatal/lingual) for all teeth.²⁴ PD was recorded as the distance from the gingival margin to the most apical part of the sulcus. Periodontal indices were calculated as a sum of mean scores by each examined tooth divided by the number of evaluated teeth. A mean of all measurements for each patient was considered.

Microbiological analysis was performed by obtaining a sample of stimulated saliva and supragingival dental plaque. The subjects had refrained from eating, drinking, brushing teeth, and chewing gum for at least 2 hours before taking the samples. Sample collection was performed between 8.30 AM and 10.30 AM. Collection of saliva and supragingival plaque samples was performed before any oral examination or manipulation so as not to disrupt the oral microbiota.

To estimate the number of colony-forming units of SM and LB per milliliter of saliva and supragingival dental plaque, Dentocult SM and LB kits (Orion Diagnostica, Espoo, Finland) were used.

Dentocult SM strip mutans was used to detect SM in stimulated saliva and plaque. Plaque samples were

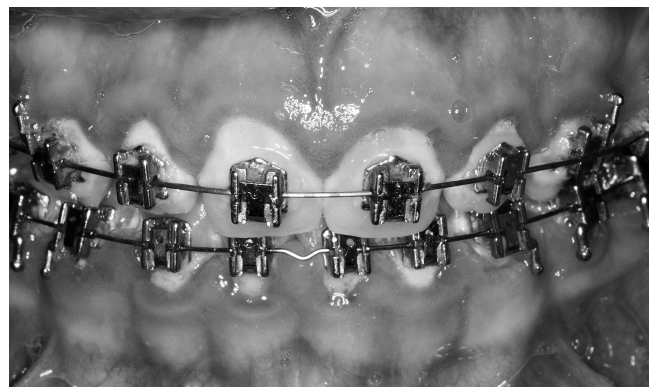


Figure 2. F1000 Self-ligating brackets.

Table 1. Demographic Characteristics of the Study Cohorts and Comparisons Between Two Groups Based on Chi-Square Test and *t*-Test^a

	CB Group (n = 20)	SLB Group (n = 20)	<i>P</i>
Female	15 (75%)	16 (80%)	NS ^b
Male	5 (25%)	4 (20%)	NS ^b
Age, y (mean ± SD)	15.05 ± 0.97	14.67 ± 1.06	NS ^c

^a CB indicates conventional brackets; SLB, self-ligating brackets; SD, standard deviation; NS, nonsignificant.

^b Chi-square test.

^c *t*-test.

collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary and mandibular lateral incisors with a sterilized different dental scaler with the same tip dimensions for each lateral tooth (\neq 8/9 Orban DE hoe scaler, Hu-Friedy, Chicago, Ill). Each sampled site was isolated from saliva contamination with cotton rolls and dried with air, and a four-pass technique was used to move the instrument tip around the circumference of the bracket at the bracket-tooth interface.

For collecting stimulated saliva, patients were instructed to chew a paraffin pellet for 1 to 5 minutes. Saliva produced during the first minute was collected for detection of SM, and then excess saliva was swallowed. The rough surface of the SM strip was pressed against the saliva remaining on the patient's tongue and removed through the gently closed lips. For LB detection, during the following 4 minutes, patients were asked to collect stimulated saliva in the mouth and to expectorate approximately 3 mL of saliva into a chilled empty Petri dish. The collected saliva was poured over both agar surfaces.

SM and LB slides were incubated at room temperature in an upright position for 7 days. The vials were labeled as per their lot numbers.

Interpretation of Results

The presence of SM was confirmed by detection of light-blue to dark-blue raised colonies on the inoculated surface of the strip. However, after incubation of LB, they are visible as white-to-transparent colonies raised on the modified Rogosa agar surface. The density of SM and LB in saliva and plaque was obtained by comparing the colony density on the test strips with the model charts.

The growth densities of SM were categorized as follows: (CFU/mL) 0 = negative or $<10^4$ CFU/mL, 1 = $<10^5$ CFU/mL, 2 = 10^5 – 10^6 CFU/mL, and 3 = $>10^6$ CFU/mL. The growth densities of LB were categorized as follows (CFU/mL): 0 = no count or 10^3 CFU/mL, 1 = 10^4 CFU/mL, 2 = 10^5 CFU/mL, and 3 = 10^6 CFU/mL.

Statistical Analysis

Bonferroni-corrected Wilcoxon signed-rank tests were used to compare the changes of the periodontal parameters in 1 month within the groups. Mann-Whitney *U*-test and Student's *t*-tests were used to compare the results between the groups. $P < .05$ was considered significant. Related tests were performed by SPSS 15.0 (SPSS Inc, Chicago, Ill). The microbiological measurements were analyzed as a two-way factorial repeated-measures design (with the factors being group and time [1, 2]) using nonparametric methods as defined by Brunner and Langer.²⁵

Demographic characteristics of the sample were investigated with conventional descriptive statistics. Differences of means were determined with the *t*-test; differences in proportion were studied with the chi-square test.

RESULTS

The distribution of gender and age did not differ between the two bracket groups (Table 1). Also, no significant differences were found in pretreatment values of the parameters between the groups (Tables 2 and 3). The results of the statistical analysis of the periodontal indexes scores and microbiological counts of the groups are shown in Tables 2 and 3.

Table 2. Periodontal Measurements at the Beginning (T_1) and 1 Month After Bonding (T_2) and Comparisons of Intra- and Intergroup Differences Based on Nonparametric and Parametric Tests^a

	CB Group (n = 20)			SLB Group (n = 20)			Intergroups
	T_1 , Median (Min–Max)	T_2 , Median (Min–Max)	<i>P</i> , T_2 - T_1	T_1 , Median (Min–Max)	T_2 , Median (Min–Max)	<i>P</i> , T_2 - T_1	<i>P</i> , T_2 - T_1
PI	0.43 (0.00–1.70)	0.93 (0.00–3.20)	.167 ^{NP}	0.30 (0.10–1.30)	1.00 (0.09–3.20)	.001 ^{NP*}	.069 ^P
GI	0.48 (0.08–1.80)	0.61 (0.09–2.10)	.472 ^{NP}	0.16 (0.09–1.60)	1.02 (0.12–2.70)	.002 ^{NP*}	.028 ^P
PD	1.77 (1.02–3.30)	2.20 (1.10–3.50)	.672 ^{NP}	1.35 (1.10–2.70)	3.00 (1.23–3.50)	$<.001$ ^{NP*}	.007 ^{NP*}

^a CB indicates conventional brackets; SLB, self-ligating brackets; PI, plaque index; GI, gingival index; PD, pocket depth; *P*, parametric; NP, nonparametric; * $P < .05$.

Table 3. Comparisons of Bacterial Colonizations at the Beginning (T_1) and 1 Month After Bonding (T_2)^a

	CB Group (n = 20)		SLB Group (n = 20)		P Group	P Time	P Group × Time
	T ₁ , Median (Min–Max)	T ₂ , Median (Min–Max)	T ₁ , Median (Min–Max)	T ₂ , Median (Min–Max)			
LB saliva	1.50 (0.00–3.00)	1.00 (0.00–3.00)	2 (0.00–3.00)	2.00 (1.00–3.00)	.488	.577	.457
SM saliva	1.00 (0.0–3.00)	2.00 (0.00–3.00)	1 (0.00–3.00)	1.00 (0.00–3.00)	.749	.341	.923
12 PlaqueSM	1.00 (0.00–3.00)	1.50 (0.00–3.00)	1 (0.00–3.00)	1.00 (0.00–3.00)	.713	.211	.161
22 PlaqueSM	1.00 (0.00–3.00)	2.00 (0.00–3.00)	1 (0.00–2.00)	1.00 (0.00–3.00)	.225	.049*	.298
32 PlaqueSM	1.00 (0.00–2.00)	1.00 (0.00–3.00)	1 (0.00–2.00)	1.00 (0.00–2.00)	.456	.002*	.670
42 PlaqueSM	0.50 (0.00–2.00)	1.00 (0.00–3.00)	0 (0.00–2.00)	1.00 (0.00–3.00)	.806	.027*	.584

^a CB indicates conventional brackets; SLB, self-ligating brackets; LB, *Lactobacillus*; SM, *Streptococcus mutans*; * $P < .05$.

Between the two groups, statistical analysis showed no difference with respect to SM and LB counts (Table 3; Figures 3 and 4).

Although during the 1-month period PI, GI, and PD scores increased in both groups, these increases were found to be significant only in the SLB group ($P < .05$). A statistically significant difference was found only in PD values between the groups ($P < .05$; Table 2).

DISCUSSION

To estimate the most hygienic bracket system, SLB and CB with stainless-steel ligature were compared in the present study, as no previous study was found in the literature. Microbial and periodontal records were obtained before upper and lower arch bonding (T_1) and 1 month after bonding (T_2), which is the time for the appointment to change the first arch wires. The study was terminated on the fourth week as longer periods of observation may affect the results because cooperation, motivation for oral hygiene, and dietary habits can change, as reported by Türkkahraman et al.⁶

Periodontal Condition

At the beginning of the study, the results showed minimum values that indicated healthy periodontia for all indices. No significant differences were found between the groups at the beginning. This result might

be due to the positive effects of oral hygiene instructions given so as to prevent the detrimental effects of orthodontic treatment on periodontal health a week before placement of the brackets.^{4,6,26,27} On the other hand, in severe crowding the hygiene supplement may not be enough; in this study, patients with minimal crowding were selected so as to eliminate the effect of crowding.

In the short time period of 1 month, placement of fixed appliances caused increased plaque accumulation, PD, and gingival inflammation in both groups, although these increases were significant only in the SLB group. An increase in PD values was found to be significantly different between the two ligation systems. This might be pseudo PD due to bracket design, size, and the proximity of the gingival margin to the bracket. The SLB used in this study were larger in size than the CB, so both the enamel surface and the distance between the brackets and gingiva were reduced, which may worsen cleanability.

The expectation of better values for periodontal indices in the SLB group was not achieved. Our finding is in accordance with the results of Van Gastel et al.,²¹ who reported significant alterations in both periodontal and microbiological parameters with the self-ligating speed brackets, and in conflict with the results of Pandis et al.,¹⁶ who found no difference in PI, GI, or PD between In-Ovation-R SLB and Microarch CB with

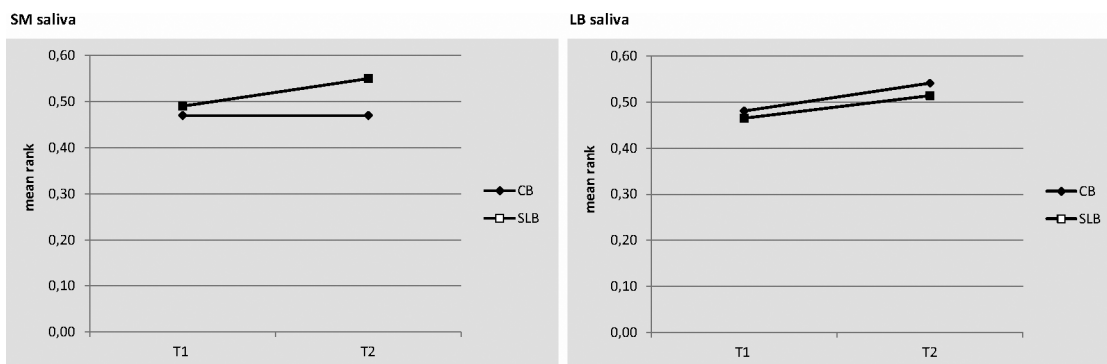


Figure 3. Mean ranks of *Streptococcus mutans* and *Lactobacillus* counts in saliva samples in the self-ligating bracket and conventional bracket groups at the beginning and 4 weeks after bonding.

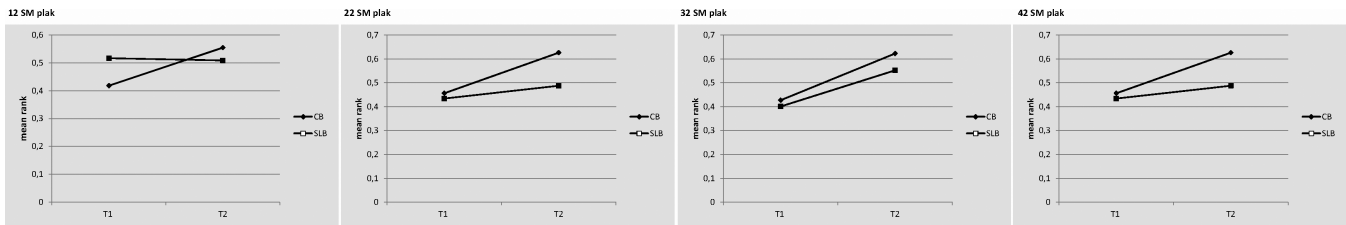


Figure 4. Mean ranks of *Streptococcus mutans* counts in plaque samples in the self-ligating bracket and conventional bracket groups at the beginning and 4 weeks after bonding.

elastomeric ligation in which periodontal evaluation was performed after completion of the alignment in the mandibular incisors to eliminate the effect of crowding. Our results may not be considered as comparable with those of Pandis et al.,¹⁶ as during the leveling phase, the oral microbiota starts to change, individual reactions begin, and the real effect of the brackets may not be evaluated properly. Our findings are also in conflict with the results of Nalçacı et al.,²² in which the Damon Q SLB group showed better values for the periodontal parameters than the group with Mini-Taurus CB with elastomeric ligation.

Microbiologic Evaluation

The number of colony-forming units of SM and LB per milliliter of saliva and plaque was estimated by Dentocult SM and LB kits as simple and easy chairside methods. The plaque samples were taken from maxillary and mandibular lateral incisors, as they were assumed to have the highest incidence of white spot formation.^{3,16,19}

In both groups, SM colonization was significantly increased in the plaque sample taken from the upper and lower left lateral incisors and lower right lateral incisor (SM 22, 32, 42). It was reported that reduced crown width and small overall teeth sizes contributed to the increase in the plaque accumulation rate on the mandibular anterior teeth.¹⁶ All patients were right-handed brushers, and the right sides may not be brushed properly.

LB and SM counts in saliva and plaque were not significantly different between the tested bracket groups. This result is in contrast with results of Pellegrini et al.¹⁹ and Mummola et al.,²³ who found lower levels of bacteria with the SLB than with elastomeric ligating brackets. In conflict with this, van Gastel et al.²¹ found higher bacterial colonization with SLB. In accordance with this, Nalçacı et al.²² and Pandis et al.²⁰ reported no difference in the levels of SM in saliva between elastomeric ligated CB and SLB. The researchers²⁰ claimed that the pretreatment levels of SM are significant predictors of SM levels after placement of brackets. In accordance with this finding, the oral hygiene instructions before the treatment might reduce the levels of SM in this study. In addition, there might be plaque

and food impactions in the space under the closing mechanism that could not be brushed by the patient without opening the mechanism. Thus, the expected advantage may not be achieved in reality.¹⁶

In the SLB group, as with the findings of Carillo et al.,¹⁸ there were no significant differences in SM and LB levels in the 1-month treatment period; however, destruction in periodontal health occurred. Another study demonstrated that 1 week following bonding, the patients with SLB presented higher colonizations of anaerobic and aerobic bacteria and increased gingival hypertrophy relative to CB, but no difference was observed in bleeding on probing between the two groups.²¹

The differences noted between the results of the present study and the others may relate to differences in sample size, sample teeth, registration times, microbiological methods, variations in the design and sizes of the brackets, methodological differences, types of ligation, bonding procedures,^{26,27} the level of oral hygiene, and the ages of the subjects.²⁸

In the light of these findings, the expected beneficial effect of the SLB is not confirmed. Even though the SLB eliminate the use of ligatures, they consist of opening and closing mechanisms, which may provide additional plaque retention sites. The results of the present study suggest that bracket type may not have a significant effect on bacterial colonization on orthodontic appliances. This might be due to oral hygiene education given at the early stages of orthodontic treatment. This result agrees with several literature reports emphasizing the main role of the oral hygiene.^{26,28}

CONCLUSIONS

- A greater increase was found in PD value in the SLB group. The SLB do not have an advantage over CB with respect to the periodontal status and colonization of SM and LB.
- Dentocult tests are simple and easy chairside methods that could be used in the orthodontic profession.

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