

## Effects of self-ligating and conventional brackets on halitosis and periodontal conditions

Emine Kaygisiz<sup>a</sup>; Fatma Deniz Uzuner<sup>b</sup>; Sema Yuksel<sup>c</sup>; Levent Taner<sup>d</sup>; Rana Çulhaoğlu<sup>e</sup>; Yasemin Sezgin<sup>f</sup>; Can Ateş<sup>g</sup>

### ABSTRACT

**Objective:** To evaluate the effects of fixed orthodontic treatment with steel-ligated conventional brackets and self-ligating brackets on halitosis and periodontal health.

**Materials and Methods:** Sixty patients, at the permanent dentition stage aged 12 to 18 years, who had Angle Class I malocclusion with mild-to-moderate crowding were randomly selected. Inclusion criteria were nonsmokers, without systematic disease, and no use of antibiotics and oral mouth rinses during the 2-month period before the study. The patients were subdivided into three groups randomly: the group treated with conventional brackets (group 1, n = 20) ligated with steel ligature wires, the group treated with self-ligating brackets (group 2, n = 20), and the control group (group 3, n = 20). The periodontal records were obtained 1 week before bonding (T1), immediately before bonding (T2), 1 week after bonding (T3), 4 weeks after bonding (T4), and 8 weeks after bonding (T5). Measurements of the control group were repeated within the same periods. The volatile sulfur components determining halitosis were measured with the Halimeter at T2, T3, T4, and T5. A two-way repeated measures of analysis of variance (ANOVA) was used to compare the groups statistically.

**Results:** No statistically significant group × time interactions were found for plaque index, gingival index, pocket depth, bleeding on probing, and halitosis, which means three independent groups change like each other by time. The risk of tongue coating index (TCI) being 2 was 10.2 times higher at T1 than at T5 ( $P < .001$ ). Therefore, the probability of higher TCI was decreased by time in all groups.

**Conclusions:** The self-ligating brackets do not have an advantage over conventional brackets with respect to periodontal status and halitosis. (*Angle Orthod.* 2015;85:468–473.)

**KEY WORDS:** Conventional brackets; Halitosis; Periodontal condition; Self-ligating brackets

<sup>a</sup> Lecturer, Department of Orthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey.

<sup>b</sup> Specialist, Department of Orthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey.

<sup>c</sup> Professor, Department of Orthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey.

<sup>d</sup> Professor, Department of Periodontology, Faculty of Dentistry, Gazi University, Ankara, Turkey.

<sup>e</sup> Research Assistant, Department of Periodontology, Faculty of Dentistry, Gazi University, Ankara, Turkey.

<sup>f</sup> Lecturer, Department of Periodontology, Faculty of Dentistry, Başkent University, Ankara, Turkey.

<sup>g</sup> Biostatistician, Department of Biostatistics, Faculty of Medicine, Ankara University, Ankara, Turkey.

Corresponding author: Dr Emine Kaygisiz, Lecturer, 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)

Accepted: June 2014. Submitted: April 2014.

Published Online: August 7, 2014

© 2015 by The EH Angle Education and Research Foundation, Inc.

### INTRODUCTION

Halitosis, which means foul breath, might be related to physiologic and/or pathologic reasons<sup>1</sup> such as ear-nose-throat diseases (chronic sinusitis, tonsillitis), gastrointestinal system diseases, diabetes mellitus, and acute rheumatic fever. Also, more frequently, halitosis can be related to intraoral factors, including especially gram-negative anaerobic microorganisms on the dental plaque, in the periodontal pockets, in saliva, and on the dorsum of the tongue.<sup>1,2</sup>

The volatile sulfur components (VSCs) consisting of hydrogen sulfide, dimethyl sulfide, and especially methyl mercaptan releasing through the proteolytic degradation of saliva, exfoliated epithelium, food debris, gingival crevicular fluid, plaque, postnasal drip, sulfur-containing amino acids, and peptides in the blood by the anaerobic microorganisms found in the oral cavity are effective on the formation of halitosis.<sup>2-4</sup> The levels of VSCs in the mouth are also influenced by

to the number and depth of periodontal pockets, the bleeding tendency of periodontal pockets, and the amount of coating on the tongue.<sup>3,5,6</sup>

The effects of orthodontic treatment on periodontal status have been evaluated in several studies.<sup>7-12</sup> The brackets and ligatures have a negative effect on natural cleaning by creating retention areas for plaques, making the mechanical cleaning of the teeth and gingiva by the tongue and lips more difficult, and increasing the viscosity of the saliva.<sup>7-9</sup> Periodontal inflammation, hyperemia, hyperplasia, and demineralization of the teeth have been found as a result of failure of ensuring adequate oral hygiene.<sup>10-13</sup> It has been stated that accumulation of plaque and the increase in bacterial count<sup>7,8,12,14</sup> and tongue coating<sup>2,6</sup> clearly are also involved in the formation of halitosis.

Determining the bracket system that causes a minimal destructive biologic effect has gained importance today. Self-ligating brackets (SLBs) were expected to have better values for periodontal status because of the lack of ligature materials and having fewer retentive sites than other bracket ligation types.<sup>7,8,15</sup> On the other hand, other researchers<sup>10</sup> mentioned that this theoretical advantage may be eliminated in reality because SLBs consist of opening and closing mechanisms that may provide additional plaque retention sites. There is only one study comparing SLBs with conventional brackets (CBs) ligated with elastic ligatures on halitosis.<sup>12</sup> However, we were unable to obtain any study comparing periodontal status and halitosis in the orthodontic treatments performed with conventional bracket systems with wire ligation and self-ligating brackets. Therefore, this prospective study was performed to investigate the effect of fixed orthodontic treatment with SLBs and steel-ligated CBs on CBs control group on halitosis, periodontal status in comparison with a control group; secondly, the investigation focused on the effect of oral hygiene, primarily tongue cleaning. The null hypothesis was that bracket type has no effect on these parameters.

## MATERIALS AND METHODS

Approval for this study was obtained from the Ethical Committee of the Dentistry Faculty of Ankara University (14.05.2012-35/1). All patients/parents were informed and their consent was given prior to entering the study.

Sixty subjects (28 female, 32 male) between the ages of 12 and 18 years were randomly selected from the patients who had applied to the Orthodontics Clinic of the University for treatment. Patients were randomly selected using these inclusion criteria: Angle Class I malocclusion with mild-to-moderate crowding at the permanent dentition stage, nonsmoker, no systemic or nasopharyngeal disease, and no use of antibiotics and oral mouth

rinses at least 2 months prior to the treatment. Patients who had mouth-breathing and a history of orthodontic treatment were excluded from the study.

The patients were subdivided into three groups randomly as follows: group 1, self-ligating brackets group (SLBs; F1000, 0.022-inch, Leone SpA, Sesto Fiorentino, Florence, Italy; n = 20); group 2, conventional brackets group ligated with steel ligatures (CBs; Avex MX, 0.022-inch, Opal Orthodontics, South Jordan, Utah; n = 20); and group 3, control group (n = 20). Mean (SD) ages for all groups were 14.7 (1.39), 14 (1.01), and 14.4 (1.46) years, respectively. The fixed orthodontic treatment of the patients in the control group was started at the end of the study.

Oral hygiene instructions were given to all patients 1 week before bonding. The method of brushing the teeth and tongue was shown with practices. Furthermore, the rules for routine hygiene and diet were delivered in writing. Patients used standardized fluoridated toothpaste and toothbrush and were asked not to use other oral hygiene supplements during the study. Patients were asked to refrain from eating foods containing garlic, onion, and spice 2 days before the measurements of halitosis and periodontal parameters. All of the measurements were carried out between 9:00 AM and 11:00 AM before breakfast. The patients were instructed to brush their teeth after dinner and refrain from eating, drinking, brushing teeth, and chewing gum until the measurement time in the morning.

A classical bonding method was used for the treatment groups. All teeth were etched with 37% phosphoric acid for 20 seconds, washed with a water spray for 10 seconds, and dried; then the brackets were bonded on the upper and lower teeth with Transbond XT adhesive (3M Unitek, Monrovia, Calif). Nickel-titanium archwires, 0.012-inch, were applied. CBs were ligated with 0.010-inch stainless steel wires.

The blinded periodontist gave oral hygiene instructions to each patient 1 week before the study. Periodontal measurements were obtained by a single calibrated periodontist 1 week before bonding (T1), immediately before the placement of upper and lower brackets (T2), 1 week after bonding (T3), 4 weeks after bonding (T4), and 8 weeks after bonding (T5). Measurements of periodontal parameters and halitosis were performed before any manipulation, such as changing the archwires, so as not to disrupt the oral microbiota. Measurements of the control group were repeated within the same periods.

Periodontal records including the plaque index (PI), gingival index (GI), bleeding on probing (BOP), and pocket depth (PD) were obtained by means of a Williams probe with 0.5-mm diameter and 1-mm calibration at four sites (mesial, midbuccal, distal, and palatal/lingual) for all teeth. PD was recorded as

**Table 1.** Comparisons of Periodontal Measurements 1 Week Before Bonding (T1), Immediately Before the Placement of Brackets (T2), and 1 Week (T3), 4 Weeks (T4), and 8 Weeks (T5) After Bonding

	Group <sup>b</sup>	T1	T2	T3	T4	T5	<i>P</i> Time	<i>P</i> Group	<i>P</i> Group × Time
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD			
PI	SLBs	0.43 ± 0.37	0.33 ± 0.43	0.38 ± 0.37	0.69 ± 0.41	0.81 ± 0.43	.004**	.406	.837
	CBs	0.45 ± 0.39	0.38 ± 0.46	0.34 ± 0.27	0.65 ± 0.45	0.78 ± 0.52			
	Control	0.36 ± 0.30	0.35 ± 0.42	0.43 ± 0.44	0.41 ± 0.52	0.63 ± 0.71			
GI	SLBs	0.20 ± 0.18	0.26 ± 0.38	0.24 ± 0.24	0.25 ± 0.17	0.58 ± 0.52	.080	.877	.346
	CBs	0.34 ± 0.41	0.40 ± 0.49	0.19 ± 0.14	0.37 ± 0.37	0.38 ± 0.35			
	Control	0.29 ± 0.28	0.24 ± 0.28	0.35 ± 0.37	0.33 ± 0.42	0.47 ± 0.51			
PD	SLBs	1.60 ± 0.48	1.48 ± 0.42	1.49 ± 0.40	1.59 ± 0.42	1.46 ± 0.23	.044*	.213	.067
	CBs	1.41 ± 0.28	1.29 ± 0.33	1.37 ± 0.20	1.33 ± 0.15	1.42 ± 0.30			
	Control	0.15 ± 0.70	1.50 ± 0.55	1.40 ± 0.52	1.48 ± 0.54	1.63 ± 0.54			
BOP	SLBs	3.95 ± 2.61	2.50 ± 1.51	3.47 ± 1.91	2.95 ± 1.48	2.34 ± 0.56	<.001***	.024*	.286
	CBs	3.26 ± 2.29	1.78 ± 0.83	2.69 ± 1.35	2.12 ± 1.18	2.11 ± 0.94			
	Control	3.03 ± 1.21	2.21 ± 0.87	2.18 ± 1.15	1.71 ± 1.18	1.97 ± 0.73			

<sup>a</sup> PI indicates plaque index; GI, gingival index; PD, pocket depth; and BOP, bleeding on probing.

<sup>b</sup> SLBs indicates self-ligating brackets; CBs: conventional brackets.

\* *P* < .05; \*\* *P* < .01; \*\*\* *P* < .001.

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 teeth divided by the number of evaluated teeth. A mean of all measurements for each patient was considered. Tongue coating index (TCI) was scored for area and thickness, and the total score was calculated as the product of both scores.<sup>16</sup>

The amount of VSCs that determines halitosis was measured with the Halimeter device (model RH17R, Interscan Corp, Chatsworth, Calif) at T2, T3, T4, and T5. The patients were asked to breathe through their noses for 3 minutes before the sampling. Then, the patients were instructed to place the disposable straw at the posterior dorsum of the tongue and not to touch the oral mucosa or the tongue. Measurement was repeated three times with 3-minute intervals. The mean value was calculated in ppb for each patient.

### Statistical Analysis

The data were statistically analyzed by using SPSS 15.0 (SPSS Inc, Chicago, Ill). The differences among the three groups, five time points, and the interaction of these two main effects were tested with two-way repeated measures of analysis of variance (ANOVA).

The ordinal logistic regression analysis by GEE (generalized estimating equations) was used to evaluate the difference among the groups in TCI value.

Demographic characteristics of the sample were investigated with odds (exp (B)) ratios, mean ± standard deviation, 95% confidence intervals, and *P* values. *P* < .05 was considered statistically significant.

### RESULTS

Power analysis showed that for a power of 0.90 at  $\alpha = .05$ , 20 patients would be required for each group. The mean age did not differ among the groups (*P* = .239). Comparisons of overall mean values for study parameters before and after treatment/observation of the groups are provided in Tables 1 through 3 and Figure 1.

No statistically significant group × time interactions were found for PI, GI, PD, BOP, and halitosis; that means three independent groups change like each other by time.

The risk of TCI being 2 was 10.2 times higher in 1 week before bonding (T1) than 2 months after bonding (T5) (*P* < .001; Table 3). Therefore, the probability of higher TCI was decreased by time in all groups.

**Table 2.** Comparison of Halitosis Immediately Before the Placement of Brackets (T2), 1 Week (T3), After Bonding, 4 Weeks (T4), After Bonding, and 8 Weeks (T5) After Bonding

	Group <sup>a</sup>	T2	T3	T4	T5	<i>P</i> Time	<i>P</i> Group	<i>P</i> Group × Time
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD			
Halitosis	SLBs	76.85 ± 64.82	62.15 ± 56.51	48.80 ± 38.94	49.20 ± 21.38	.425	.211	.344
	CBs	53.20 ± 41.19	64.90 ± 40.90	49.15 ± 36.19	43.10 ± 31.05			
	Control	34.87 ± 34.28	43.93 ± 39.75	42.93 ± 38.00	47.40 ± 25.17			

<sup>a</sup> SLBs indicates self-ligating brackets; CBs: conventional brackets.

**Table 3.** Comparison of Tongue Coating Index 1 Week Before Bonding (T1), Immediately Before the Placement of Brackets (T2), and 1 Week (T3), 4 Weeks (T4), and 8 Weeks (T5) After Bonding

Group <sup>a</sup>	T1 Median (min, max)	T2 Median (min, max)	T3 Median (min, max)	T4 Median (min, max)	T5 Median (min, max)	Odds (exp (B))	95% CI	P
SLBs	1 (0, 2)	0.5 (0, 1)	1 (0, 2)	0 (0, 1)	0 (0, 1)	1		
CBs	1 (0, 2)	1 (0, 2)	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.627	0.390–1.440	.387
Control	1 (0, 2)	0 (0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.750	0.336–1.170	.143

<sup>a</sup> SLBs indicates self-ligating brackets; CBs: conventional brackets.

## DISCUSSION

The effects of fixed orthodontic treatments on periodontal status have been mostly evaluated in the literature<sup>7–12</sup>; however, there are limited numbers of studies considering the effects of bracket types on halitosis and periodontal status.<sup>11,12</sup> And, of these, none of them compared steel-ligated with self-ligating brackets. Therefore, the goal of our study was to determine the bracket system that will cause minimal destructive effect.

Although studies with longer periods of follow-up are required to evaluate the effects of bracket systems on halitosis and periodontal indices more accurately, this study was terminated in the eighth week as the longer periods of observation may affect the results because cooperation, motivation for oral hygiene, and dietary habits can change.<sup>17</sup>

Previous studies indicated that increase in tongue coating and periodontal diseases were two major halitosis-inducing factors by creating areas allowing the overgrowth of anaerobic bacteria.<sup>18–20</sup> For this reason, in this study patients were instructed to brush both teeth and tongue.

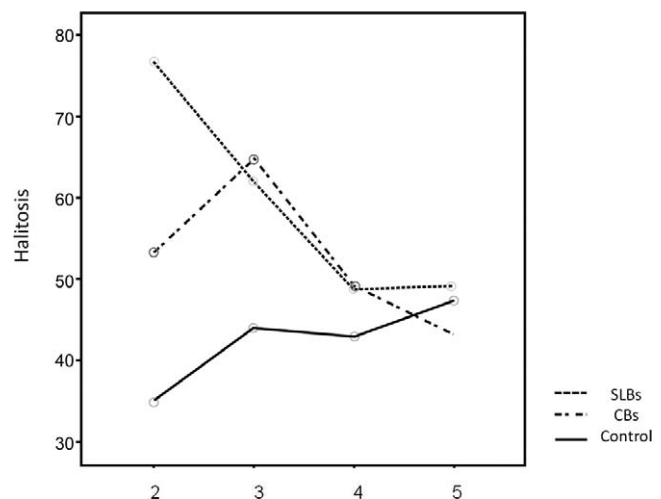
There were no significant differences in pretreatment values (T2; 1 week after oral hygiene instructions) of the PI, GI, PD, BOP, and halitosis parameters between the groups. Before bonding, all periodontal indices showed minimum values in all groups. This might be due to the positive effects of oral hygiene instructions given 1 week before the beginning of the study, as also mentioned in other studies.<sup>15,17</sup> On the other hand, the hygiene supplement may not be enough in patients with severe crowding; in this study, patients with mild-to-moderate crowding were selected so as to eliminate the effect of crowding.

The amount of VSCs (ppb) in the breath for the diagnosis of halitosis was measured by the Halimeter. The Halimeter is preferred because it provides an objective measurement, is portable, does not require experienced personnel, has low probability of cross-infection, and has 1- to 2-minute intervals between measurements.<sup>21,22</sup> Organoleptic measurements were not preferred due to being subjective and having cross-infection risks.<sup>23</sup> The gas chromatography device was

also not preferred because it is expensive and complex and requires an experienced physician.<sup>2</sup> However, it has been reported that measuring only the VSCs would not be sufficient in determining halitosis and that the organoleptic method related to other gases would give more definitive data.<sup>23</sup> However, recent studies have shown that data obtained with the Halimeter are consistent with data found with organoleptic measurements.<sup>2,22,23,24</sup>

The placement of SLBs and CBs both caused increased plaque accumulation. PI scores increased significantly both in 4 weeks and in 8 weeks after bonding in all groups. However, no significant differences were found between the groups for overall time. In accordance with this, Pandis et al.<sup>25</sup> defined no difference in PI between SLBs and CBs with elastomeric ligation. Our result is in contrast with the findings of some researchers<sup>7,8,12,17</sup> who defined that elastomeric ligated CBs cause more plaque accumulation and periodontal inflammation than SLBs.

There were decreases in PD values in treatment groups, while an increase was seen in the control group between T1 and T3. But, no significant differences were found in PD between the groups for overall time points. BOP scores were higher in the SLBs group than other groups for overall time.



**Figure 1.** The changes of halitosis in the self-ligating brackets, conventional brackets, and control groups.

The results of this study showed no significant differences in PI, GI, PD, and BOP values changes during the treatment/observation time among the groups. This may be attributed to the effect of an oral hygiene supplement given before the treatment/observation and motivation of the patients. It can also be attributed to the Hawthorne effect<sup>26</sup> (patients' awareness of being examined and evaluated).

It seems that the SLBs do not have any benefit over steel-ligated CBs. Even though the SLBs eliminate the use of ligatures, they consist of opening and closing mechanisms, which may provide additional plaque retention sites.<sup>10</sup>

Although nonsignificant decreases were found 4 weeks and 8 weeks after bonding in the SLBs and steel-ligated CBs groups and increase in the control group, no significant differences were found between the groups for overall time in halitosis. According to the manufacturer's manual, 160 ppb is the cognitive threshold value used to discriminate patients with or without noticeable halitosis, so the patients in this study were all in the weak stage (0–100 ppb) throughout the study.

These results of this study are in conflict with the findings of Babacan et al.<sup>11</sup> who reported increases in halitosis, PI, and GI values 1 week after bonding with elastomeric ligated CBs, and who reported that the PI and GI values remained stable 4 weeks later, while the nonsignificant increase in halitosis continued. In contrast with this, Nalçacı et al.<sup>12</sup> found that periodontal parameters and halitosis values were higher in the CBs group than in the SLBs group. These different results may be related to the tongue cleaning, as the effect of tongue coating on halitosis was not taken into consideration in the previous orthodontic studies. The mechanical cleaning of the tongue was reported to reduce both the amount of coating and the number of bacteria, thereby improving oral malodor effectively.<sup>2,6,19</sup> Just the contrary, some researchers claimed that the reduction in microbial load on the tongue after cleaning is negligible.<sup>27,28</sup>

In this study, the risk of TCI being 2 was 10.2 times higher in 1 week before bonding (T1) than 2 months after bonding (T5). Therefore, the probability of higher TCI was decreased by time in all groups. This result showed that tongue cleaning may positively affect the halitosis scores.

The different results noted between this study and others may also relate to variations in the design and sizes of the brackets, methodologic differences, types of ligation, bonding procedures,<sup>29,30</sup> and the level of oral hygiene of the subjects.<sup>31</sup>

In light of these findings, the null hypothesis could not be rejected because no differences were found between F1000 SLBs and Avex MX CBs on periodontal

status and halitosis. Rather than the bracket types oral hygiene especially, brushing both teeth and tongue seems to be more effective in minimizing halitosis which was in consistent with the studies emphasizing the main role of the oral hygiene.<sup>29,31</sup>

## CONCLUSIONS

- The SLBs do not have an advantage over CBs with respect to periodontal status and halitosis.
- Rather than periodontal status, the tongue coating might have more effect on halitosis.

## REFERENCES

1. Newman MG, van Winkelhof AJ. *Antibiotic and Antimicrobial Use in Dental Practice*. 2nd ed. London, UK: Quintessence; 2000.
2. Nalçacı R, Sönmez IS. Evaluation of oral malodor in children. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2008;106:384–388.
3. Quirynen M, Zhao H, van Steenberghe D. Review of the treatment strategies for oral malodour. *Clin Oral Invest*. 2002;6:1–10.
4. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol*. 1977;48:13–20.
5. Persson S. Hydrogen sulfide and methyl mercaptan in periodontal pockets. *Oral Microbiol Immunol*. 1992;7:378–379.
6. De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *J Am Dent Assoc*. 1995;126:1384–1393.
7. Lara-Carrillo E, Montiel-Bastida NM, Sánchez-Pérez L, Alanís-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of *Streptococcus mutans* and *Lactobacillus*. *Med Oral Patol Oral Cir Bucal*. 2010;15:924–929.
8. Pellegrini P, Sauerwein R, Finlayson T, et al. Plaque retention by self-ligating vs elastomeric orthodontic brackets: quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. *Am J Orthod Dentofacial Orthop*. 2009;135:426.e1–e9.
9. Scheie AA, Arneberg P, Krogstad AO. Effect of orthodontic treatment on prevalence of *Streptococcus mutans* in plaque and saliva. *Scand J Dent Res*. 1984;92:211–217.
10. Pandis N, Vlachopoulos K, Polychronopoulou A, Madianos P, Eliades T. Periodontal condition of the mandibular anterior dentition in patients with conventional and self-ligating brackets. *Orthod Craniofac Res*. 2008;11:211–215.
11. Babacan H, Sokucu O, Marakoglu I, Ozdemir H, Nalçacı R. Effect of fixed appliances on oral malodor. *Am J Orthod Dentofacial Orthop*. 2011;139:351–355.
12. Nalçacı R, Ozat Y, Cokakoğlu S, Türkrahman H, Onal S, Kaya S. Effect of bracket type on halitosis, periodontal status, and microbial colonization. *Angle Orthod*. 2014;84:479–485.
13. Mitchell L. Decalcification during orthodontic treatment with fixed appliances: an overview. *Br J Orthod*. 1992;19:199–205.
14. Tonzetich J. Production and origin of oral malodor: a review of mechanisms and methods of analysis. *J Periodontol*. 1977;48:13–20.

15. Qgaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am J Orthod.* 1998;94:68–73.
16. Vandekerckhove B, Van den Velde S, De Smit M, et al. Clinical reliability of non-organoleptic oral malodour measurements. *J Clin Periodontol.* 2009;36:964–969.
17. Türkahraman H, Sayin O, Bozkurt Y, Yetkin Z, Kaya S, Onal S. Archwire ligation techniques, microbial colonization, and periodontal status in orthodontically treated patients. *Angle Orthod.* 2005;75:231–236.
18. Takeshita T, Suzuki N, Nakano Y, et al. Relationship between oral malodor and the global composition of indigenous bacterial populations in saliva. *Appl Environ Microbiol.* 2010;76:2806–2814.
19. Miyazaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. *J Periodontol.* 1995;66:679–684.
20. Yaegaki K, Sanada K. Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *J Periodontol Res.* 1992;27:233–238.
21. Doty RL, Green PA, Ram C, Yankel SL. Communication of gender from human breath odors: relationship to perceived intensity and pleasantness. *Horm Behav.* 1982;16:13–22.
22. Rosenberg M, Kulkarni GV, Bosy A, McCulloch CA. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. *J Dent Res.* 1991;70:1436–1440.
23. Murata T, Yamaga T, Iida T, Miyazaki H, Yaegaki K. Classification and examination of halitosis. *Int Dent J.* 2002;52(suppl 3):181–186.
24. Van den Velde S, van Steenberghe D, Van Hee P, Quirynen M. Detection of odorous compounds in breath. *J Dent Res.* 2009;88:285–289.
25. Pandis N, Papaioannou W, Kontou E, Nakou M, Makou M, Eliades T. Salivary *Streptococcus mutans* levels in patients with conventional and self-ligating brackets. *Eur J Orthod.* 2010;32:94–99.
26. Feil PH, Grauer JS, Gadbury-Amyot CC, Kula K, McCunniff MD. Intentional use of the Hawthorne effect to improve oral hygiene compliance in orthodontic patients. *J Dent Educ.* 2002;66:1129–1135.
27. Menon MV, Coykendall AL. Effect of tongue scraping. *J Dent Res.* 1994;73:1492.
28. Quirynen M, Avontrodt P, Soers C, Zhao H, Pauwels M, van Steenberghe D. Impact of tongue cleansers on coating, microbial load and taste sensation in periodontitis free subjects. *J Clin Periodontol.* 2004;31:506–510.
29. Amasyali M, Enhos S, Uysal T, Saygun I, Kilic A, Bedir O. Effect of a self-etching adhesive containing an antibacterial monomer on clinical periodontal parameters and subgingival microbiologic composition in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2011;140:e147–e153.
30. Sukontapatipark W, El-Agroudi M, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. *Eur J Orthod.* 2001;23:475–484.
31. Smiech-Slomkowska G, Jablonska-Zrobek J. The effect of oral health education on dental plaque development and the level of caries-related *Streptococcus mutans* and *Lactobacillus* spp. *Eur J Orthod.* 2007;29:157–160.