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Original article

Adhesion of mutans streptococci to self-ligating ceramic brackets: *in vivo* quantitative analysis with real-time polymerase chain reaction

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Summary

Objective: To analyze *in vivo* mutans streptococci (MS) adhesion to self-ligating ceramic brackets [Clarity-SL (CSL) and Clippy-C (CC)] and the relationships between bacterial adhesion and oral hygiene indices.

Materials and methods: Four central incisor brackets from the maxilla and mandible were collected from 40 patients (20 patients per each bracket type) at debonding immediately after plaque and gingival indices were measured. Adhesions of *Streptococcus mutans*, *S. sobrinus*, and total bacteria were quantitatively determined using real-time polymerase chain reaction after genomic DNA was extracted. Factorial analysis of variance was used to analyze bacterial adhesion to the brackets with respect to the bracket type and jaw position. Correlation coefficients were calculated to determine the relationships of bacterial adhesion to oral hygiene indices.

Results: Adhesion of total bacteria and *S. mutans* to CSL was higher than that to CC ($P < 0.001$). Adhesion of total bacteria to the mandibular brackets was higher than that to the maxillary ones ($P < 0.001$), while adhesion of *S. mutans* to the maxillary brackets were higher than that in the mandibular ones ($P < 0.001$). In particular, the proportion of *S. mutans* to total bacteria in CSL was higher than CC ($P < 0.05$) in the maxillary anterior teeth ($P < 0.001$). There were no significant differences in adhesion of *S. sobrinus* between the brackets and jaw positions. Interestingly, no significant relationships were found between bacterial adhesions and oral hygiene indices.

Limitations: Complex bracket configurations may significantly influence bacterial adhesion to orthodontic brackets. Further *in vivo* study using bracket raw materials will help to define the relationships between bacteria adhesion and enamel demineralization.

Conclusions: Because oral hygiene indices were not significantly correlated with adhesions of MS to self-ligating ceramic brackets, careful examinations around the brackets should be needed to prevent enamel demineralization, regardless of oral hygiene status.

Introduction

Enamel demineralization frequently occurs in patients with fixed orthodontic treatment (1, 2). Orthodontic brackets play a significant role in enamel demineralization (3), because they provide additional retentive surfaces for oral bacteria and make traditional oral hygiene procedures more difficult (4). As a result, brackets may change the oral environment around orthodontic appliances, including increased plaque accumulation, increased acidogenic bacteria, and

decreased pH (5). The environmental changes around brackets can contribute significantly to developing enamel demineralization (6).

Mutans streptococci (MS) are generally considered the major causative organism of enamel demineralization (7). Among them, *Streptococcus mutans* and *S. sobrinus* are most frequently found in the human oral cavity (8). Many studies have investigated MS adhesion to orthodontic brackets (9–12), suggesting that MS adhesion to orthodontic brackets varies according to bracket materials and types (9) Recently, self-ligating brackets have been introduced to the market (13). Although some studies have sought to assess differences in the adhesion of oral bacteria between self-ligating and conventional brackets (11, 12), few studies have analyzed the differences in MS adhesion to self-ligating ceramic brackets, specifically in an *in vivo* setting. In addition, the effects of MS adhesion to self-ligating brackets on oral hygiene indices remain unknown.

Various methods have been used to identify MS, including colony morphology on mitis-salivarius bacitracin agar, biochemical and immunological tests, and polymerase chain reaction (PCR). Recently, real-time PCR has emerged as a more rapid and sensitive method of quantifying specific bacterial species with minimal chances of contamination (14). In addition, the sample for real-time PCR can be stored for a long time in a freezer in a non-living state, which helps researchers to manage many samples easily in an *in vivo* study (14).

The purpose of this prospective *in vivo* study was to quantitatively analyze differences in adhesion of *S. mutans*, *S. sobrinus*, and total bacteria to two different self-ligating ceramic brackets using real-time PCR and to investigate the relationships between bacterial adhesions and oral hygiene indices.

Materials and methods

The study population initially comprised adult subjects who were poised to finish orthodontic treatment with fixed appliances. Inclusion criteria were 1) men and women greater than 19 and 17 years of age, respectively, 2) a longer than 12-month treatment period (average 20.2 months), and 3) the following two self-ligating ceramic bracket types with a 0.022-inch slot: Clarity-SL (CSL) (3M Unitek, Monrovia, California, USA) and Clippy-C (CC) (Tomy, Tokyo, Japan). Exclusion criteria were 1) any systemic disease, 2) active carious lesions, 3) poor oral hygiene with simplified oral hygiene index (OHI-S) over 3.0 (15), 4) topical fluoride application (except for fluoridated dentifrice) or antibacterial therapy within 6 months, and 5) subjects wearing elastomeric chains prior to sample collection. After the sample size was determined from information of previously reported articles (11, 12), the brackets were collected from 40 subjects (20 subjects per each bracket type; 11 men and 29 women, mean ages 23.4 years). All subjects signed informed consent forms and the Institutional Review Board of the University Hospital approved the study protocol.

All subjects were asked to refrain from eating, teeth brushing, or mouth rinsing at least 2 hours before debonding. At the time of debonding, the left and right central incisor brackets of the maxilla and mandible for four brackets per patient were collected (total 160 brackets) immediately after OHI-S plaque index (PI), and gingival index (GI) of the same teeth were examined by a single investigator (16). OHI-S was a representation of oral hygiene status calculated using debris and calculus deposition from two anterior and four posterior teeth (15). PI and GI are categorical scales. In the case of PI, 0 indicates no plaque accumulation; 1 indicates invisible plaque adhering to the tooth that can be scraped using only a probe; 2 indicates moderate accumulation which can be seen with the naked eye; and 3 indicates abundant accumulation. In the case of GI, 0 indicates no abnormal status of gingiva; 1 indicates mild inflammation, but no bleeding on probing; 2 indicates moderate inflammation with bleeding on probing; and 3 indicates severe inflammation with tendency to spontaneously bleed (16).

Each bracket was washed with 1.0 ml cold phosphate-buffered saline (PBS, pH = 7.4) to remove unbound bacteria. The bracket was placed in the round tube with 2.0 ml cold PBS and homogenized by sonication using three 30 seconds pulses with 30 seconds intermittent cooling stages in a chilled ice box (17). They were centrifuged at 13 000 revolutions per minute for 10 minutes after removing brackets. The pellet was then subject to DNA extraction.

Bacterial chromosomal DNA was extracted using a CellEase Bacteria II Genomic DNA Extraction Kit (Biocosm, Osaka, Japan) as previously described (17). Specific known primers that amplify the dextranase gene of *S. mutans* and *S. sobrinus* were designed from the *gtfB* and *gtfU* genes, respectively (Table 1). A conserved sequence in the 16S ribosomal RNA was selected to quantify the number of total bacteria (17). All primers were commercially synthesized (Takara-Korea, Seoul, Korea). When testing primer specificities using 11 strains of MS and other 5 Gram-positive and 11 Gram-negative species (Table 2), amplified DNA was only detected in the target bacteria.

DNA was extracted from *S. mutans* UA159 and *S. sobrinus* SL1 to generate standard curves. DNA concentration was estimated by absorbance at 260 nm and the amount of bacterial DNA in the samples was extrapolated from the standard curve as previously described (18).

Real-time PCR was performed using the Bio-Rad iQ5 system (Bio-Rad, Hercules, California, USA). The reaction mixtures contained 2 µl purified DNA from the samples, 100 pmol primer, and 10 µl 2x iQ SYBR Green Supermix (Bio-Rad). Distilled water was added to a final volume of 20 µl. The samples were subjected to an initial amplification for 30 seconds at 94°C, 40 cycles of denaturation for 20 seconds at 95°C, primer annealing for 45 seconds at 60°C, and extension for 10 seconds at 60°C. All data including amounts of total bacteria, *S. mutans*, and *S. sobrinus* were analyzed with iQ5 Optical System Software (Bio-Rad). All the experiments for quantifying bacteria were performed in triplicate and independently repeated twice.

Two-way factorial analysis of variance (ANOVA) was used to determine significant differences in the amounts of bacterial

Table 1. Specific primers used in the study.

Primer		Sequence	Position	Amplicon size
Universal	Forward	5′-TGGAGCATGTGGTTTAATTCGA-3′	930–951	160 bp
	Reverse	5′-TGCGGGACTTAACCCAACA-3′	1089–1071	
gtfB	Forward	5′-CTACACTTTCGGGTGGCTTG-3′	794–813	261 bp
	Reverse	5′-GAAGCTTTTCACCATTAGAAGCTG-3′	1054–1031	
gtfU	Forward	5′-AAAACATTGGGTTACGATTGCG-3′	39–60	156 bp
	Reverse	5′-CGTCATTGGTAGTAGCCTGA-3′	193–951	

adhesion between bracket types and jaw positions after quantifying amounts and transforming logarithm of the amounts of *S. mutans*, *S. sobrinus*, and total bacteria. Values were considered statistically significant when *P* was less than 0.05 after Tukey's honestly significant difference (HSD) correction. Spearman's rank correlation coefficients were calculated to determine the relationships of bacterial adhesions with oral hygiene indices on the same tooth. All statistical analysis was performed using SAS v 9.3 software (SAS Institute Inc., Cary, North Carolina, USA).

Results

Table 3 shows the amount of adhesion of total bacteria, *S. mutans*, and *S. sobrinus*, and the proportion of *S. mutans* and *S. sobrinus* to total bacteria with respect to the bracket type and jaw position. The results of factorial ANOVA indicate that main factors, such as the bracket type and jaw position, have significant effects on bacterial adhesion without significant interactions (data not shown).

The adhesions of total bacteria and *S. mutans* to CSL were higher than that to CC ($P < 0.001$). The proportion of *S. mutans* to total bacteria in CSL was also higher than in CC ($P < 0.05$). However, there were no significant differences in adhesion of *S. sobrinus* and the proportion of *S. sobrinus* to total bacteria between two brackets ($P > 0.05$).

Although the amount of adhesion of total bacteria was higher in the mandible than in the maxilla ($P < 0.001$), the amount of adhesion of *S. mutans* and the proportion of *S. mutans* to total bacteria was higher in the maxilla than in the mandible ($P < 0.001$). There were no significant differences in amount of adhesion of *S. sobrinus* and the proportion of *S. sobrinus* to total bacteria between the maxilla and mandible ($P > 0.05$).

Table 4 shows the relationships of adhesion amounts of total bacteria, *S. mutans*, and *S. sobrinus*, and the proportion of *S. mutans* and *S. sobrinus* to total bacteria with PI or GI. None of the variables were significantly correlated with PI or GI ($P > 0.05$).

Table 5 shows bracket configurations used in this study. CSL is longer and higher than CC. In addition, CSL is wider than CC when considering additional nitinol clips which attach to CSL. The both maxillary brackets are larger than the mandibular ones except for height.

Discussion

Generally, the amount of adhesion of *S. mutans* was higher than that of *S. sobrinus* (Table 3). In particular, there were obvious differences between the proportions of *S. mutans* and *S. sobrinus* to total bacteria. Our results are consistent with previous studies which demonstrated that the binding affinity of *S. mutans* to orthodontic brackets was higher than that of *S. sobrinus* (9, 10). This is also explained by the fact that *S. mutans* is isolated more frequently than *S. sobrinus* in the oral cavity (19).

This study showed that bacterial adhesion to self-ligating ceramic brackets is different between the two brackets (Table 3). The adhesion of total bacteria to CSL was significantly higher than the adhesion to CC. The adhesion pattern of *S. mutans* was similar to that

Table 2. Bacteria used for primer specificity testing.

Mutans streptococci	Other Gram-positive bacteria	Gram-negative bacteria
Streptococcus cricetus E49	*S. gordonii* DL1	*Escherichia coli* DH5a
S. ratti BHT 9	*S. gordonii* M5	*Actinobacillus actinomycetemcomitans* 33385
S. ratti FA-1F	*S. mitis* 9811	*Actinomyces naslundii* 12104
S. mutans Ingbritt	*S. sanguinis* 10558	*A. viscosus* 19226
S. mutans LM7	*S. sanguinis* MPC1	*Fusobacterium nucleatum* 10953
S. UA159		*F. nucleatum* 27067
S. mutans OMZ175		*Lactobacillus acidophilis* 5906
S. mutans GH5IS		*Prevotella intermedia* 25611
S. sobrinus B13		*Porphyromonas gingivalis* 381
S. sobrinus SL1		*P. gingivalis* W50
S. sobrinus 6715		*Bacteroides intermedius* 532-70A

Table 3. Adhesion amounts of total bacteria, *Streptococcus mutans*, and *S. sobrinus*, the proportion of *S. mutans* and *S. sobrinus* to total bacteria with respect to two self-ligating ceramic brackets [Clarity-SL (CSL) and Clippy-C (CC)], and different jaw positions [maxilla (Mx) and mandible (Mn)]. The unit of total bacteria, *S. mutans*, and *S. sobrinus* is the cell number in logarithm. Adhesion is expressed as the mean ± standard deviation.

	CSL (n = 80)		CC (n = 80)		
	Mx (n = 40)	Mn (n = 40)	Mx (n = 40)	Mn (n = 40)	Significance†
Total bacteria (log_{10}/ml)	8.91 ± 0.44	9.01 ± 0.39	8.68 ± 0.29	8.93 ± 0.42	CSL > CC** Mx < Mn**
S. mutans (log_{10}/ml)	6.62 ± 1.23	5.94 ± 1.26	5.79 ± 1.18	5.49 ± 0.94	CSL > CC** Mx > Mn**
S. sobrinus (log_{10}/ml)	5.02 ± 0.47	4.90 ± 0.48	4.94 ± 0.55	4.97 ± 0.63	NS
S. mutans/total bacteria (%)	4.37 ± 6.35	1.06 ± 1.81	2.22 ± 6.47	0.75 ± 3.21	CSL > CC* Mx > Mn**
S. sobrinus/total bacteria (%)	0.03 ± 0.08	0.02 ± 0.03	0.14 ± 0.50	0.39 ± 3.19	NS

ANOVA, analysis of variance.

†Two-way ANOVA was used to determine the significant differences in bacterial adhesions between bracket types and jaw positions with Tukey's HSD correction: NS, not significant; *$P < 0.05$; **$P < 0.001$.

Table 4. Statistical significances (*P* values) and correlation coefficient values of Spearman's rank correlation analysis between bacterial adhesion and plaque index (PI) or gingival index (GI).

	PI		GI	
	P value	Correlation coefficient	*P* value	Correlation coefficient
Total bacteria	0.86	0.008	0.78	−0.012
Streptococcus mutans	0.07	−0.081	0.11	−0.071
S. sobrinus	0.09	0.077	0.53	−0.028
S. mutans/total bacteria	0.10	−0.074	0.25	−0.051
S. sobrinus/total bacteria	0.11	0.071	0.76	−0.014

Table 5. The size information (width × length × height, mm) of self-ligating ceramic brackets [Clarity-SL (CSL) and Clippy-C (CC)] according to jaw position.

		Maxillary central incisor	Mandibular central incisor
CSL	Base	3.35 × 4.20 × 2.70	2.05 × 3.90 × 3.00
	Wing	3.30 × 4.05 × 2.70 (5.15)*	2.00 × 3.90 × 3.00 (3.60)*
CC	Base	4.05 × 3.90 × 2.65	3.40 × 3.65 × 2.90
	Wing	3.40 × 4.05 × 2.65	3.00 × 4.00 × 2.90

The size was measured using an Absolute Digimatic calipers (Mitutoyo, Kanagawa, Japan).

*The bracket width included additional nitinol clips.

of total bacteria and the adhesion of *S. mutans* to CSL was also higher than that of CC. The higher bacterial adhesion to CSL might be explained by the difference in bracket configuration. While CC is self-ligating ceramic brackets with a cover, CSL is a slot-opened bracket with additional nitinol clips at both ends without a cover. The cover of CC may hinder bracket slots, which impedes further bacterial adhesion around bracket slots. In addition, the complex bracket configuration of CSL with additional nitinol clips may provide suitable niches for bacterial accumulation. Bracket size may also influence bacterial adhesion to the brackets (Table 5). CSL is larger than CC, especially in length and height. The width of CSL is also larger than that of CC when considering additional nitinol clips. The bulky and complex bracket configuration may enhance bacterial adhesions to CSL. Because CSL has a similar shape to other conventional non-self-ligating brackets except for nitinol clips, this study suggest a possible benefit of self-ligating brackets with caps or covers for maintaining oral hygiene. Further *in vivo* studies will be needed to elucidate the reason for the differences in bacterial adhesion to different brackets.

There were significant differences in bacterial adhesion to self-ligating ceramic brackets between the maxilla and mandible (Table 3). The adhesion of total bacteria to the mandibular brackets was higher than adhesion to the maxillary brackets. According to a previous study (20), dental plaque tends to accumulate on the mandibular dentition compared to the maxillary dentition. Clinically, plaque may be more visible on mandibular incisors than maxillary incisors in orthodontic patients (21). As more plaque accumulates, more bacteria can be available for detection. This may be due to the specific role of saliva in the bacterial adhesion and plaque accumulation. Salivary pellicles formed on the tooth surfaces significantly contribute to plaque accumulation by providing specific receptors for primary colonizers (22). Therefore, increased adhesion of total bacteria to the mandibular brackets may be associated with the location of the major salivary gland near the mandibular incisors.

On the contrary to total bacterial adhesion, the adhesion of *S. mutans* to the maxillary brackets was higher than adhesion to the mandibular brackets (Table 3). This result is consistent with a previous study which showed that *S. mutans* is more prevalent in the anterior brackets of the maxilla compared to the mandible (9). Saliva, which can act as a buffer which is resistant to changes in pH (23), may be more abundant around mandibular incisors than maxillary incisors due to the presence of the major salivary gland. Therefore, maxillary incisors may provide a more favourable environment for aciduric and acidogenic bacteria, such as *S. mutans*. The difference in bracket size may partly influence the difference in *S. mutans* adhesion to the brackets of the maxilla and mandible (Table 5). The maxillary brackets are generally larger than the mandibular brackets. Brackets with larger surface areas may facilitate increased *S. mutans* adhesion.

The proportion of MS to total bacteria in plaques can be more important in evaluating risk for dental caries than the absolute numbers of MS alone (24). The present study showed that the proportion of *S. mutans* to total bacteria was higher in CSL than in CC, and higher in the maxillary brackets than the mandibular ones (Table 3). Higher proportion of *S. mutans* to total bacteria in maxillary anterior brackets can partly explain why white spot lesions are more frequent on the maxillary incisors than the mandibular incisors (25). These findings suggest that the patients wearing self-ligating ceramic brackets, especially CSL than CC, should be carefully monitored during orthodontic treatment in order to prevent enamel demineralization around brackets, specifically on the maxillary incisors.

Unlike *S. mutans*, the adhesion and proportion of *S. sobrinus* showed no significant differences between the brackets and between jaw positions. Bacteria in MS group, which have similar features such as acidogenic and aciduric characteristics, can exhibit different adhesion abilities for the brackets (9, 10). The insignificant differences in adhesion pattern are due to the fact that the amount of *S. sobrinus* adhesion to the brackets was smaller than the amount of *S. mutans* adhesion (Table 3). As mentioned above, this may be partly due to the fact that *S. sobrinus* is isolated less frequently than *S. mutans* in the oral cavity (19).

Adhesion of total bacteria, *S. mutans*, and *S. sobrinus* to the self-ligating ceramic brackets and the proportion of *S. mutans*, and *S. sobrinus* to total bacteria were not significantly associated with oral hygiene indices on the tooth such as PI or GI (Table 4). Our findings are consistent with a previous study which showed that the prevalence of MS was not associated with oral hygiene status (10). Oral hygiene may not be directly correlated with the amount of adhesion of total bacteria and MS to the brackets. Although PI and GI have the merit of simplicity and wide usage throughout dentistry, they may be less appropriate for the categorization of plaques on bracketed teeth because they are general indices that account for common patterns of plaque accumulation on teeth without brackets (26).

When we analyzed the amounts of bacterial adhesion to lateral incisor brackets, we observed the same adhesion patterns of

S. mutans, *S. sobrinus*, and total bacteria to central incisor brackets according to the bracket type and jaw position (data not shown). Therefore, this study suggests that orthodontists should carefully examine the maxillary anterior regions in patients with self-ligating ceramic brackets, specifically CSL, to prevent enamel demineralization, regardless of oral hygiene status.

Limitations

Although we demonstrated differences in MS adhesion to self-ligating ceramic brackets, higher MS adhesion to a specific bracket may not directly indicate the risk for enamel demineralization during orthodontic treatment. Further *in vivo* study using bracket raw materials will be needed to verify the relationships between the amounts of cariogenic bacteria attached to brackets and enamel demineralization. Nevertheless, the results of this study may provide valuable information on the managements of orthodontic patients with self-ligating ceramic brackets.

Conclusions

This *in vivo* study was performed to evaluate the adhesion level of *S. mutans*, *S. Sobrinus*, and total bacteria to two different self-ligating ceramic brackets using real-time PCR. This study revealed that the oral bacteria including MS can adhere to self-ligating ceramic brackets. In particular, CSL showed the higher adhesion in both total bacteria and *S. mutans*, which may be due to its bulky size and complex configuration. Contrary to patterns of total bacterial adhesion, *S. mutans* adhered to maxillary incisor brackets more than to mandibular incisor brackets. Oral hygiene indices were not significantly correlated with adhesions of total bacteria and MS to the brackets.

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References

1. Benson, P.E., Pender, N. and Higham, S.M. (2003) Quantifying enamel demineralization from teeth with orthodontic brackets--a comparison of two methods. Part 1: repeatability and agreement. *European Journal of Orthodontics*, 25, 149–158.
2. Cildir, S.K., Germec, D., Sandalli, N., Ozdemir, F.I., Arun, T., Twetman, S. and Caglar, E. (2009) Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. *European Journal of Orthodontics*, 31, 407–411.
3. Pandis, N., Papaioannou, W., Kontou, E., Nakou, M., Makou, M. and Eliades, T. (2010) Salivary *Streptococcus mutans* levels in patients with conventional and self-ligating brackets. *European Journal of Orthodontics*, 32, 94–99.
4. Smiech-Slomkowska, G. and Jablonska-Zrobek, J. (2007) The effect of oral health education on dental plaque development and the level of caries-related *Streptococcus mutans* and *Lactobacillus spp*. *European Journal of Orthodontics*, 29, 157–160.
5. Tufekci, E., Dixon, J.S., Gunsolley, J.C. and Lindauer, S.J. (2011) Prevalence of white spot lesions during orthodontic treatment with fixed appliances. *Angle Orthodontist*, 81, 206–210.
6. O'Reilly, M.M. and Featherstone, J.D. (1987) Demineralization and remineralization around orthodontic appliances: an in vivo study. *American Journal of Orthodontics and Dentofacial Orthopedics*, 92, 33–40.
7. Featherstone, J.D. (2000) The science and practice of caries prevention. *Journal of American Dental Association*, 131, 887–899.
8. Babaahmady, K.G., Challacombe, S.J., Marsh, P.D. and Newman, H.N. (1998) Ecological study of *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus spp*. at sub-sites from approximal dental plaque from children. *Caries Research*, 32, 51–58.
9. Ahn, S.J., Lee, S.J., Lim, B.S. and Nahm, D.S. (2007) Quantitative determination of adhesion patterns of cariogenic streptococci to various orthodontic brackets. *American Journal of Orthodontics and Dentofacial Orthopedics*, 132, 815–821.
10. Ahn, S.J., Lim, B.S. and Lee, S.J. (2007) Prevalence of cariogenic streptococci on incisor brackets detected by polymerase chain reaction. *American Journal of Orthodontics and Dentofacial Orthopedics*, 131, 736–741.
11. Baka, Z.M., Basciftci, F.A. and Arslan, U. (2013) Effects of 2 bracket and ligation types on plaque retention: a quantitative microbiologic analysis with real-time polymerase chain reaction. *American Journal of Orthodontics and Dentofacial Orthopedics*, 144, 260–267.
12. Pellegrini, P., Sauerwein, R., Finlayson, T., McLeod, J., Covell, D.A., Jr, Maier, T. and Machida, C.A. (2009) Plaque retention by self-ligating vs elastomeric orthodontic brackets: quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. *American Journal of Orthodontics and Dentofacial Orthopedics*, 135, 426. e1–e9.
13. Othman, S.A., Mansor, N. and Saub, R. (2014) Randomized controlled clinical trial of oral health-related quality of life in patients wearing conventional and self-ligating brackets. *Korean Journal of Orthodontics*, 44, 168–176.
14. Yano, A., Kaneko, N., Ida, H., Yamaguchi, T. and Hanada, N. (2002) Real-time PCR for quantification of *Streptococcus mutans*. *FEMS Microbiology Letters*, 217, 23–30.
15. Greene, J.C. and Vermillion, J.R. (1964) The simplified oral hygiene index. *Journal of American Dental Association*, 68, 7–13.
16. Loe, H. (1967) The gingival index, the plaque Index and the retention index systems. *Journal of Periodontology*, 38(Suppl), 610–616.
17. Jung, W.S., Kim, H., Park, S.Y., Cho, E.J. and Ahn, S.J. (2014) Quantitative analysis of changes in salivary mutans streptococci after orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, 145, 603–609.
18. Yin, J.L., Shackel, N.A., Zekry, A., McGuinness, P.H., Richards, C., Putten, K.V., McCaughan, G.W., Eris, J.M. and Bishop, G.A. (2001) Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for measurement of cytokine and growth factor mRNA expression with fluorogenic probes or SYBR Green I. *Immunology and Cell Biology*, 79, 213–221.
19. Wennerholm, K. and Emilson, C.G. (1995) Sucrose retention and colonization by mutans streptococci at different sites of the dentition. *Caries Research*, 29, 396–401.
20. Furuichi, Y., Lindhe, J., Ramberg, P. and Volpe, A.R. (1992) Patterns of de novo plaque formation in the human dentition. *Journal of Clinical Periodontology*, 19, 423–433.
21. Zachrisson, S. and Zachrisson, B.U. (1972) Gingival condition associated with orthodontic treatment. *Angle Orthodontist*, 42, 26–34.
22. Nishihara, T. and Koseki, T. (2004) Microbial etiology of periodontitis. *Periodontology 2000*, 36, 14–26.
23. Bardow, A., Moe, D., Nyvad, B. and Nauntofte, B. (2000) The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. *Archives of Oral Biology*, 45, 1–12.
24. Choi, E.J., Lee, S.H. and Kim, Y.J. (2009) Quantitative real-time polymerase chain reaction for *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque samples and its association with early childhood caries. *International Journal of Paediatric Dentistry*, 19, 141–147.
25. Julien, K.C., Buschang, P.H. and Campbell, P.M. (2013) Prevalence of white spot lesion formation during orthodontic treatment. *Angle Orthodontist*, 83, 641–647.
26. Al-Anezi, S.A. and Harradine, N.W. (2012) Quantifying plaque during orthodontic treatment. *Angle Orthodontist*, 82, 748–753.