Comparative evaluation of salivary microbial levels and periodontal status of patients wearing fixed and removable orthodontic retainers

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Introduction: The purpose of this study was to compare and evaluate salivary microbial levels and periodontal status in patients using a fixed lingual retainer, a removable vacuum-formed retainer, or a Hawley retainer after orthodontic treatment with fixed appliances. **Methods:** Forty-five patients who finished their orthodontic treatment with fixed appliances and were about to start the retention phase were randomly divided into the following 3 groups of 15 individuals each: the fixed lingual retainer group, the vacuum-formed retainer group, and the Hawley retainer group. Periodontal measurements, such as the plaque index, gingival index, probing depth, and bleeding on probing, were obtained at the following 4 time points: at debonding (T0) and 1 week (T1), 5 weeks (T2), and 13 weeks (T3) after debonding. Saliva samples were collected 3 times in total: at T0, T2, and T3. A quantitative analysis for *Streptococcus mutans* and *Lactobacillus casei* was performed with the use of real-time polymerase chain reaction. The Kruskal-Wallis test and 1-way analysis of variance were used for the statistical comparisons of the groups. **Results:** No statistically significant difference in salivary *S mutans* and *L casei* levels was found among the 3 groups ($P > 0.05$). They showed no statistically significant differences in plaque index, gingival index, bleeding on probing, and probing depth values ($P > 0.05$). All periodontal parameters showed statistically significant decreases from T0 to T3 in all 3 groups ($P < 0.001$). The *S mutans* and *L casei* levels were decreased significantly from T2 to T3 in the lingual retainer and Hawley retainer groups, whereas they decreased significantly from T0 to T3 in the vacuum-formed retainer group. **Conclusions:** Fixed and removable orthodontic retainers do not differ in salivary *S mutans* and *L casei* levels and periodontal status. With all retainers, regardless of whether they are fixed or removable, oral hygiene improved after orthodontic treatment with fixed appliances. (Am J Orthod Dentofacial Orthop 2019;156:186-92)

In orthodontic patients, fixed or removable orthodontic appliances cause worsening of oral hygiene.[1] These appliances change the microbial flora of the oral cavity, which may lead to dental caries, white spot lesions, and gingival inflammation.[2,3] Mutans streptococci (predominantly *S mutans*) play a major role in the initiation of carious lesions, whereas lactobacilli may be more prominent in their progression.[4] Studies have shown that cariogenic microorganisms, including *S mutans* and lactobacilli, increase in the dental plaque and saliva of patients after the bonding of orthodontic appliances.[3,5] Therefore, knowing the bacterial changes in patients receiving orthodontic treatment, including the retention phase, is important.

The retention phase, in which dental movements are stabilized after active treatment, is very important for the success of orthodontic treatment. Numerous fixed and removable retention methods are routinely used in clinical practice, and all of them have many advantages and disadvantages. Fixed retainers make providing oral hygiene procedures difficult and support the accumulation of plaque and calculus,[6] whereas removable retainers cover all tooth surfaces and prevent the flushing effect of saliva.[7]

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Most studies in the literature have evaluated the changes in *S mutans* and *Lactobacillus* levels during orthodontic treatment with fixed or removable appliances[1,3,5,8,9]; however, microbiologic data on the retention phase after active orthodontic treatment are limited.[7,10-12] In the literature, when microbiologic data are evaluated in the retention phase, no comparison is made with different retention methods, and the effect of a single retainer is not evaluated. To our knowledge, the present study is the first to compare the effects of a fixed lingual retainer, a removable vacuum-formed retainer, and a Hawley retainer on oral microbial flora and periodontal status.

Nowadays, real-time polymerase chain reaction (PCR) is commonly used as a fast and precise method for determining specific bacterial species and their quantities.[13] Basing on these pieces of information, the purpose of this study was to compare and evaluate periodontal status and salivary microbial levels by means of real-time PCR in patients using a fixed lingual retainer, a removable vacuum-formed retainer, or a Hawley retainer, all of which are frequently used in the retention phase. The null hypothesis of our study was that no difference exists between removable and fixed orthodontic retainers in terms of their effects on periodontal status and salivary microbial levels.

MATERIAL AND METHODS

This study was carried out on 45 individuals (11 male and 34 female) who finished their orthodontic treatment with fixed appliances and were about to start the retention phase at the Selcuk University Faculty of Dentistry Department of Orthodontics. Their mean age was 15.2 ± 2.1 years (range, 11.0-20.9 years). The size of the population was predetermined by means of power analysis in G*Power version 3.0.10 software (Franz Faul, Universitat Kiel, Germany). On the basis of a 1:1 ratio between groups, a total sample size of 45 patients would give more than 80% power (actual power 0.8173) for the 3 groups and 3 repeated measurements to detect significant differences with a 0.40 effect size at the $\alpha = 0.05$ significance level. The material of this study consisted of clinical periodontal index examinations and the saliva samples taken from our patients at different times.

Ethics Committee approval of the Selcuk University Faculty of Medicine (number 2016/109) and consent forms from the patients and their parents were obtained for this study.

The following criteria were considered in the selection of patients: no caries-active individuals, no antibiotic use within the past 3 months, no smoking, no periodontal or systemic disorder, and no prosthesis in the mouth.

Forty-five patients who met the inclusion criteria and were about to complete their orthodontic treatment were randomly divided into 3 groups with 15 members each: the fixed lingual retainer group, the vacuum-formed retainer group, and the Hawley retainer group. All patients were provided with standard oral hygiene training, along with scaling and polishing procedures, 1 week before the debonding. They were instructed to brush their teeth 3 times a day. They were given standardized toothpastes and toothbrushes and asked not to use any other oral care products throughout the study. After the debonding, one of the retainers routinely used in the retention phase was applied randomly, and the retention procedure was initiated. The fixed lingual retainers were bonded to the lingual surfaces of the 6 anterior teeth of both the maxilla and the mandible by adapting a 0.0215-inch 5-stranded wire (Penta One; Masel Orthodontics, Carlsbad, Calif). The vacuum-formed retainers (Ortho Technology, Lutz, Fla) covering all tooth surfaces up to the gingival margin were applied to both the maxilla and the mandible. The Hawley retainers, consisting of polymethylmethacrylate-based acrylic (Imicryl O-80; Konya, Turkey), and a 0.7-mm-thick stainless steel wire were fabricated by means of heat cure and applied to both the maxilla and the mandible. The patients were instructed to wear their removable retainers all day, except during meals, and to brush them after toothbrushing.

The measurements of the plaque index, gingival index, probing depth, and bleeding on probing, which are the clinical parameters of dental plaque accumulation, were performed at debonding (T0) and 1 week (T1), 5 weeks (T2), and 13 weeks (T3) after debonding. These periodontal measurements were recorded for 6 anterior teeth at 6 sites per tooth for the plaque index, probing depth, and bleeding on probing and at 4 sites per tooth for the gingival index. The periodontal evaluation was carried out by the same trained clinician (A.K.E.) with the use of a periodontal probe (Hu-Friedy, Chicago, Ill).

Saliva samples were collected at T0, T2, and T3. The microbiologic samples were taken before the clinical periodontal evaluation by the same clinician (A.K.E.). The patients were asked not to eat or drink anything, nor to brush their teeth or gargle up to 2 hours before the saliva sample was taken. Approximately 5 mL of saliva samples was collected in sterile tubes and stored at −80°C for the real-time PCR analysis.

Table I. Primer and probe sequences for real-time polymerase chain reaction

Bacteria	*Primers and probe*	*Sequence*	*Replicated base pairs*
S mutans	Forward	5′-CCGGTGACGGCAAGCTAA-3′	114
	Reverse	5′-TCATGGAGGCGAGTTGCA-3′	
	Probe	FAM-5′-CTCTGAAAGCCGATCTCAGTTCGGATTG-TAMRA-3′	
L casei	Forward	5′-CTATAAGTAAGCTTTGATCCGGAGATTT-3′	132
	Reverse	5′-CTTCCTGCCGGTACTGAGATGT-3′	
	Probe	FAM-5′-ACAAGCTATGAATTCACTATGC-TAMRA-3′	

Bacterial DNA was extracted from the saliva samples with the use of an extraction kit (DNeasy Blood and Tissue kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The primers and probes used for the detection and quantification of the cariogenic microorganisms are listed in Table I. The fluorescent dyes at the 5′ and 3′ ends of the probe were 6-carboxyfluorescein (FAM; reporter) and 6-carboxytetramethylrhodamine (TAMRA; quencher), respectively. The species-specific probe and primer sets were designed based on the variable regions of the 16S ribosomal RNAs of *S mutans*[14] and *L casei*,[15] as previously described. A universal bacterial primer pair was used to detect DNA from all eubacterial species in the samples. All primers and probes were checked for possible cross-hybridization with bacterial genes with the use of a database similarity search program.

A quantitative assay was achieved by cloning plasmids containing the amplified region of each target bacterium with cloning procedures (Topo-XL PCR Cloning; Invitrogen, Carlsbad, Calif). Each PCR amplicon for *S mutans* and *L casei* was individually inserted into a separate plasmid vector; the recombinant vectors were transformed into One Shot Chemically Competent *Escherichia coli* (Invitrogen). The plasmids were purified with the use of a plasmid purification kit (Plasmid DNA Purification; Macherey-Nagel, Düren, Germany). Quantification of the target DNA was achieved with serial 10-fold dilutions from 10^x to 10^y of the plasmid copies from the previously quantified standards, specifically from 10^2 to 10^6 for *L casei* and from 10^3 to 10^6 for *S mutans*. The plasmid standards and clinical samples were run in duplicate, and the average values were used for calculating the bacterial loads.

Real-time PCR reactions were performed with the use of Lightcycler TaqMan master mix (Roche Applied Science, Mannheim, Germany). The samples were assayed in duplicate in a 20-μL reaction mixture containing 5 μL template DNA, 4 μL master mix at 5× concentration, 10 pmol forward primer and reverse primer, and 5 pmol probe (Synthesis Report; Metabion, Martinsried, Germany). The cycling conditions used were as follows: 95°C for 10 minutes, followed by 40 cycles at 95°C for 30 seconds, 60°C for 1 minute, 40°C for 40 seconds each, and extension at 72°C for 1 minute. The results were analyzed on the thermal cycler instrument software (Lightcycler, version 1.2; Roche Applied Science) by quantitatively analyzing the fluorescence emissions. All PCRs were performed in duplicate.

Statistical analysis

All analyses were carried out with the SPSS package for statistical analysis (version 21.0; IBM, Armonk, NY). The normality assumption required for statistical analysis was checked by means of the Shapiro-Wilk normality test. The homogeneities of the group variances were analyzed by means of the Levene test. The normality test showed that the clinical periodontal findings did not exhibit a normal distribution, whereas the microbiologic findings exhibited a normal distribution.

In the statistical evaluation of clinical periodontal findings, the Kruskal-Wallis test was used for intergroup comparison, whereas the Friedman and Nemenyi post hoc tests were used for intragroup comparison. In the statistical evaluation of microbiologic findings, 1-way analysis of variance (ANOVA) was used for intergroup comparison, whereas repeated-measures ANOVA and Bonferroni post hoc tests were used for intragroup comparison (Bonferroni adjustment for *S mutans*: $P < 0.025$ was used in the lingual retainer group and $P < 0.05$ in the Hawley and vacuum-retainer groups; for *L casei*: $P < 0.025$ in the lingual retainer group and $P < 0.016$ in the Hawley and vacuum-retainer groups). The correlations between clinical periodontal findings and microbiologic findings were analyzed by means of the Spearman rho correlation test. Log 10 transformation was performed on the microbiologic data so that the distribution was normalized and the variance was stabilized. Values were considered to be statistically significant at $P < 0.05$.

RESULTS

The mean values of the periodontal measurements and the salivary bacterial counts at T0 were not

Table II. Means, standard deviations, and statistical comparisons of the bacterial counts (log 10)

			T0		T2		T3		P value*		
Bacteria	Group	n	Mean	SD	Mean	SD	Mean	SD	T0-T2	T0-T3	T2-T3
S mutans	LR	15	4.92	1.41	5.01	1.3	4.44	1.17	1	0.139	0.019‡
	Hawley	15	4.48	0.95	4.93	1.14	4	1	0.296	0.142	0.029‡
	VFR	15	4.94	1.28	4.65	1.27	4.23	1.09	0.519	0.034‡	0.192
	P value†		0.522		0.718		0.558				
L casei	LR	15	4.37	1.19	4.69	1.4	3.95	1.34	0.411	0.19	0.017‡
	Hawley	15	4.04	1.41	4.42	1.16	3.59	1.26	0.083	0.325	0.011‡
	VFR	15	4.33	0.85	4.44	1.59	3.47	1.35	1.000	0.004‡	0.011‡
	P value†		0.708		0.846		0.587				

LR, Lingual retainer; *VFR,* vacuum-formed retainer.
*Bonferroni multiple comparison test result (intragroup comparisons); †One-way analysis of variance (ANOVA) test result (intergroup comparison); ‡Significant difference.

statistically significant among the 3 groups. The means, standard deviations, and statistical comparisons of the bacterial counts for the 3 retention groups are presented in Table II. The *S mutans* and *L casei* levels were decreased significantly from T2 to T3 in the lingual retainer and Hawley retainer groups, whereas in the vacuum-formed retainer group the *S mutans* levels decreased significantly from T0 to T3 and the *L casei* levels decreased significantly from T0 to T3 and from T2 to T3 ($P < 0.05$). After debonding (from T0 to T2), all retention groups showed insignificant increases in the *S mutans* and *L casei* levels, except for the *S mutans* level of the vacuum-formed retainer group ($P > 0.05$). No statistically significant difference in the salivary *S mutans* and *L casei* levels was found among the 3 groups ($P > 0.05$).

The means, standard deviations, and statistical comparisons of the periodontal measurements for the 3 retention groups are presented in Table III. The plaque index, gingival index, probing depth, and bleeding on probing measurements showed a statistically significant decrease for the vacuum-formed retainer group from T0 to T2, from T0 to T3, and from T1 to T3. The same measurements showed a statistically significant decrease for the lingual retainer and Hawley retainer groups from T0 to T2 and from T0 to T3. The 3 groups showed no statistically significant differences in the plaque index, gingival index, bleeding on probing, and probing depth values ($P > 0.05$). On the basis of the results, the null hypothesis of this study was accepted.

A significant positive correlation was found between all clinical periodontal measurements ($P < 0.001$), whereas no significant correlations were observed between the *S mutans* and *L casei* levels. A positive low correlation was found between the *S mutans* level and probing depth ($r = 0.184$; $P = 0.033$). The other correlations were not statistically significant.

DISCUSSION

The changes in microbial and periodontal parameters during orthodontic treatment with the use of fixed or removable appliances have been evaluated in many studies.[1,3,5,8,9] However, the changes in microbial parameters during the retention phase have been explored in only a few studies.[7,10-12] Those studies usually focused on the effect of a single retainer, and no comparisons of different retainers were made. Although some studies compared the effects of fixed and removable retainers on periodontal health,[16,17] whether the prevalence of dental caries and periodontal outcomes differed between a fixed retainer and a removable retainer was uncertain. Therefore, in the present study, we aimed to compare and evaluate periodontal status and salivary microbial levels with the use of real-time PCR in patients using a fixed lingual retainer, a removable vacuum-formed retainer, or a Hawley retainer, all of which are frequently used in the retention phase.

Orthodontic retainers maintain stability after orthodontic treatment, and they can be fixed or removable. Fixed retainers are made of wire with different sizes and materials, and they are bonded to the teeth on the lingual side with composite resin. Fixed retainers with varying extensions generally include the 6 anterior teeth. Removable retainers generally cover all the teeth and can be vacuum-formed or in the form of an acrylic splint with clasps.[18] Fixed lingual retainers may lead to plaque accumulation in the teeth because ensuring oral hygiene may be difficult. The use of some oral hygiene instruments, such as dental floss, is also challenging.[19] This raises the need for comparing fixed and removable retainers regarding their effects on oral microbial flora and periodontal status. The lingual retainer that is routinely used in our clinic was administered to patients for fixed retention, and the Hawley and vacuum-formed

Table III. Means, standard deviations, and statistical comparisons of the periodontal measurements

Clinical periodontal index	Group	n	T0		T1		T2		T3		P value*					
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	T0-T1	T0-T2	T0-T3	T1-T2	T1-T3	T2-T3
Probing depth	LR	15	1.96	0.36	1.7	0.32	1.66	0.3	1.59	0.27	0.053	<0.001‡	<0.001‡	0.325	0.004‡	0.325
	Hawley	15	1.76	0.39	1.53	0.4	1.47	0.4	1.46	0.38	0.076	<0.001‡	<0.001‡	0.013‡	0.064	0.942
	VFR	15	1.81	0.36	1.61	0.34	1.56	0.31	1.52	0.3	0.016	<0.001‡	<0.001‡	0.714	0.03‡	0.325
	P value†		0.364		0.877		0.678		0.879							
Gingival index	LR	15	0.99	0.51	0.3	0.22	0.24	0.21	0.15	0.14	0.030	<0.001‡	<0.001‡	0.49	0.013‡	0.364
	Hawley	15	0.89	0.65	0.43	0.45	0.17	0.2	0.1	0.15	0.146	<0.001‡	<0.001‡	0.125	0.013‡	0.831
	VFR	15	0.99	0.61	0.4	0.41	0.29	0.42	0.19	0.38	0.091	<0.001‡	<0.001‡	0.125	0.002‡	0.535
	P value†		0.741		0.868		0.474		0.75							
Bleeding on probing	LR	15	0.22	0.12	0.04	0.06	0.01	0.02	0.01	0.02	0.005	<0.001‡	<0.001‡	0.831	0.714	0.997
	Hawley	15	0.28	0.13	0.08	0.09	0.06	0.09	0.04	0.05	0.002	<0.001‡	<0.001‡	0.942	0.714	0.96
	VFR	15	0.26	0.15	0.11	0.09	0.05	0.08	0.02	0.06	0.091	<0.001‡	<0.001‡	0.146	0.03‡	0.92
	P value†		0.72		0.088		0.286		0.147							
Plaque index	LR	15	0.44	0.25	0.16	0.11	0.07	0.1	0.03	0.07	0.091	<0.001‡	<0.001‡	0.076	0.013‡	0.92
	Hawley	15	0.5	0.37	0.13	0.11	0.07	0.06	0.01	0.02	0.03	0.002‡	<0.001‡	0.865	0.053	0.289
	VFR	15	0.6	0.44	0.23	0.27	0.13	0.21	0.08	0.17	0.076	<0.001‡	<0.001‡	0.125	0.005‡	0.67
	P value†		0.646		0.498		0.653		0.307							

LR, Lingual retainer; *VFR,* vacuum-formed retainer.
*Nemenyi multiple comparison test result (intragroup comparisons); †Kruskal-Wallis test result (intergroup comparisons); ‡Significant difference.

retainers were administered to patients for removable retention. The Hawley retainer covers only the lingual or palatinal part of the teeth with its acrylic plate.[19] The vacuum-formed retainer prevents the flushing effect of saliva by covering the entire surface of the teeth.[7] For this reason, 2 different groups were created for removable retention, and these 2 different appliances were used.

According to the study by Jung et al,[12] a statistically significant decrease was observed in the total amount of bacteria 5 weeks after debonding. Statistically significant increases were found in *S mutans* and *S sobrinus* levels 5 and 13 weeks after debonding, respectively, and the ratio of *S mutans* and *S sobrinus* bacteria to the total amount of bacteria peaked by showing a statistically significant increase 13 weeks after debonding. On the basis of these results, in the present study, saliva samples were taken at debonding (T0) and 5 (T2) and 13 (T3) weeks after debonding.

Page and Schroeder[20] reported that an early gingival lesion was formed as a result of plaque accumulation for 7-9 days. In the study by Jung et al,[12] oral hygiene index values showed a statistically significant decrease 1 week after debonding. In other words, the oral hygiene of patients improved significantly 1 week after debonding. Accordingly, in the present study, the clinical periodontal evaluation was performed at debonding (T0) and 1 (T1), 5 (T2), and 13 (T3) weeks after debonding.

Acid-producing cariogenic bacteria, especially *S mutans* and *Lactobacillus*, cause enamel demineralization.[21] These cariogenic bacteria are particularly emphasized in studies of oral hygiene during and after orthodontic treatment.[3,5,7-10,12] In the present study, *S mutans* and *L casei* levels in saliva and their changes during retention phase were examined. The measurement of bacteria in saliva is an easy-to-implement and noninvasive procedure. It also provides an overall assessment without being limited to certain regions within the oral cavity compared with bacteria measurement in the plaque.[12] A positive correlation was reported among the amount of cariogenic bacteria in the saliva, the amount of plaques, and, consequently, the risk of developing caries.[22-24]

Fixed orthodontic treatment makes ensuring oral hygiene difficult, and it affects the oral flora by increasing the retentive areas for bacterial accumulation. For this reason, oral hygiene improves with the removal of orthodontic appliances.[25] Jung et al[12] reported that oral hygiene index values sharply decreased 1 week after debonding and maintained low scores during their study. Similarly, Kim et al[11] evaluated the simplified

oral hygiene index, plaque index, and gingival index of patients with maxillary wraparound and mandibular Hawley retainers after debonding. They reported that all periodontal parameters were significantly decreased immediately after debonding and oral hygiene was improved. In our study, the plaque index, gingival index, probing depth, and bleeding on probing measurements showed a statistically significant decrease for all groups from T0 to T3 (T0 > T2,T3). In the Hawley retainer group, a statistically significant decrease was found in the plaque index and bleeding on probing measurements 1 week after debonding (T0 > T1). Although oral hygiene improvement took a longer time in the vacuum-formed retainer and lingual retainer groups than in the Hawley retainer group, no statistically significant differences were observed between groups. Similarly to our study, Heier et al[16] evaluated the periodontal implications of removable or fixed retainers at baseline (just before debonding) and at 1, 3, and 6 months later, reporting a generalized improvement in gingival health in both fixed and removable retainer groups. Storey et al[17] evaluated the plaque index, gingival index, and calculus index of upper and lower lingual retainers versus upper and lower vacuum-formed retainers over 12 months; they reported that compared with vacuum-formed retainers, after 12 months of retention lingual retainers were associated with a greater accumulation of plaque and calculus and gingival inflammation. They suggested an initial improvement in oral hygiene after the removal of fixed appliances for both groups, but with time the periodontal measurements of all patients worsened. We conclude that the different results of these studies, which compared removable and fixed retainers, are due to the short follow-up period involved and the increased motivation of patients who were given toothpastes and toothbrushes during their oral hygiene training.

Rosenbloom and Tinanoff[10] examined *S mutans* levels in saliva before, during, and after orthodontic treatment. According to their results, the salivary *S mutans* level showed a significant increase during the treatment, but it decreased during the retention phase after the treatment and reached the same level as before the orthodontic treatment. The samples in the retention phase were collected after a period of 6-15 weeks after all bands and orthodontic attachments were removed. In concordance with that study, we found a statistically significant decrease in the salivary *S mutans* level in the samples taken at week 13 after debonding in the vacuum-formed retainer group. However, in the Hawley and lingual retainer groups, the *S mutans* levels increased at week 5 after debonding and decreased at week 13.

In the study by Jung et al,[12] 43 of the 58 subjects received a maxillary-mandibular removable retainer and lingual retainer together, and 15 subjects received only a maxillary-mandibular removable retainer. A statistically significant decrease in the total bacterial levels in saliva was observed at 5 weeks after the debonding in the study, with improvement in oral hygiene. However, for the *S mutans* and *S. sobrinus* levels, statistically significant increases were observed at 5 and 13 weeks after debonding. Thirteen weeks after the debonding, the ratio of *S mutans* and *S. sobrinus* bacteria to the total bacteria showed a statistically significant increase and peaked. Similarly, in our study, increase in the *S mutans* levels for the Hawley and lingual retainer groups and increase in the *L casei* levels for all retention groups were found 5 weeks after debonding. However, these increases were not statistically significant. In contrast to the results of Jung et al,[12] the *S mutans* level in our study significantly decreased from the 5th week to the 13th week after debonding for the Hawley and lingual retainer groups, whereas a significant decrease occurred at 13 weeks after debonding for the vacuum-retainer group. The reason for the difference in outcomes may be that Jung et al[12] did not group the patients by the retention appliances they use, and accordingly, some patients were using only removable retainers whereas others were using removable and fixed retainers together.

No statistically significant difference was found in the salivary *S mutans* and *L casei* levels between fixed and removable retainers. Although some studies compared the effects of fixed and removable retainers on periodontal health, evidence comparing the effects of these retainers on oral microbial flora is lacking. For this reason, we have not been able to discuss our results.

This study provides information on the quantitative analysis of *S mutans* and *L casei* with the use of real-time PCR and the periodontal status of patients using a fixed lingual retainer, a removable vacuum-formed retainer, or a Hawley retainer. That the number of patients was limited and the collection time of the saliva samples was limited to 3 owing to financial constraints are the limitations of this study. The diverse results of studies comparing removable and fixed retainers are due to different sample sizes (adequate sample size versus limited sample size) and microbiologic comparative methods (taking plaque samples from many areas versus few areas). Further studies covering a longer period with a longer sample collection time and more patients are needed, as well as studies comparing microbiologic data by taking plaque samples from different areas.

CONCLUSIONS

The following conclusions can be drawn within the limitations of this in vivo study: (1) Fixed and removable orthodontic retainers do not differ in their salivary *S mutans* and *L casei* levels and periodontal status; and (2) with all retainers, regardless of whether they are fixed or removable, oral hygiene is improved after orthodontic treatment with fixed appliances.

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