Original Article

Microbiologic changes in subgingival plaque before and during the early period of orthodontic treatment

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ABSTRACT

Objective: To evaluate changes in subgingival microbiota before and during the leveling and alignment orthodontic stage using the polymerase chain reaction (PCR) method.

Materials and Methods: Thirty orthodontic patients (17 females and 13 males; aged 16.7 ± 6.5 y) were included in this study. Subgingival microbial samples were taken from the disto-buccal gingival crevice of the left upper central incisors, the left lower central incisors, the mesio-buccal gingival crevice of the left upper first molars, and the left lower first molars, at four different times: at baseline, before placement of orthodontic appliances (T1), and 1 week (T2), 3 months (T3), and 6 months after placement of orthodontic appliances (T4). DNA was extracted from the samples, and the 16S rRNA-based PCR detection method was used to determine the prevalence of *Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Campylobactor rectus, Eikenella corrodens, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens,* and *Treponema denticola,* which are considered as putative periodontopathogens.

Results: The frequency of *T forsythia, C rectus, and P nigrescens* significantly increased after placement of orthodontic appliances. For the other species, the frequency tended to increase but no statistically significant difference was noted. The frequency of the change, representing microorganisms not existing at T1 but newly developing at T2, T3, and T4, was higher at the molars than at the incisors.

Conclusion: The placement of orthodontic appliances affects the subgingival microbial composition even during the early period of orthodontic treatment, increasing the prevalence of periodontopathogens, especially in the molar region. (*Angle Orthod.* 2012;82:254–260.)

KEY WORDS: Periodontopathogens; Gingivitis; PCR; Orthodontic appliance

INTRODUCTION

During orthodontic therapy, orthodontists occasionally are confronted with gingival hyperplasia and bleeding on probing.[1–4] Orthodontic attachments accelerate the accumulation of bacterial plaque through difficulties in maintaining adequate oral hygiene.[2,4,5] Deep probing depth caused by gingival hyperplasia offers a favorable environment for periodontopathogenic anaerobic bacteria. However, it is not yet clear whether the periodontal changes that occur during orthodontic treatment are permanent. Some studies have reported that patients lose significantly more clinical attachment during orthodontic treatment than untreated controls.[3] In contrast, other studies have

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found that overall gingival changes produced by orthodontic appliances are transient with no permanent damage to the periodontal tissues.[2,6,7] These contradictory findings probably stem from differences in clinical assessment techniques used to evaluate the periodontal attachment level, observation time, and prophylactic measures used during orthodontic treatment, as well as from individual differences in the immune response against pathogenic bacteria. It has been recognized that bacterial plaque is the major etiologic factor in the initiation and progression of gingivitis and periodontitis.[5,8] Thus x-ray and clinical assessment such as pocket depth, attachment level, and bleeding on probing are not sufficient to describe the effects of orthodontic therapy on periodontal tissues.

Some studies have reported that the placement of orthodontic appliances affects the subgingival microbial composition, increasing the prevalence of periodontopathogens.[5,9–13] However, these earlier studies had some weak points, such as (1) a small number of species were tested,[12,13] (2) a cross-sectional study design was used,[10–12] and (3) only a few studies used a polymerase chain reaction (PCR) method, which has greater sensitivity and specificity compared with other microbiologic identification techniques such as cell culturing and the DNA probe method, especially in the detection of anaerobic bacteria.[9–11] In addition, very little information is available on the change in diversity of microorganisms according to specific sites of the dental arch.

The purpose of this study was to evaluate longitudinal changes that occur in the subgingival microbiota during the leveling and alignment orthodontic stage using the PCR method. In addition, we evaluated various strains and the effects of tooth position on growth. The null hypothesis of this study is that no differences in the frequency of periodontopathogens exist between the periods of the initial leveling stage of orthodontic treatment.

MATERIALS AND METHODS

Subjects and Clinical Procedures

Thirty subjects (17 females and 13 males; aged 16.7 ± 6.5 y [mean ± SD]) were consecutively selected from among patients who arrived for orthodontic treatment at the Department of Orthodontics, Gangneung-Wonju National University Dental Hospital, Gangneung, South Korea. The study design was approved by the Ethics Committee (IRB2010-9-3). Subjects were enrolled according to the following criteria: (1) no known systemic disease; (2) no periodontal treatment within 6 months; (3) no use of antimicrobial and anti-inflammatory drugs within 3 months before the baseline examination; and (4) a healthy gingival condition defined as a periodontal probing depth of less than 4 mm, and a plaque index[14] and a gingival index[14] less than 1. The gingival condition was assessed by one periodontist.

All subjects received orthodontic therapy with fixed buccal appliances. For orthodontic treatment, metal brackets (Victory, 3M Unitek, Monrovia, Calif) were bonded indirectly with composite resin (Excel, Reliance Orthodontic Products Inc, Itasca, Ill) onto incisors, and premolars and bands (Seamless bands, Tomy, Tokyo, Japan) were cemented with polyacid-modified composite resin (Ultra Band-Lok, Reliance Orthodontic Products Inc, Itasca, Ill) onto molars within 1 week after the bonding procedure. The arch wires were ligated using stainless steel ligatures. No dropouts were noted during the observation periods.

Subgingival microbial samples were collected from the disto-buccal gingival crevice of the left upper central incisors (U1) and the left lower central incisors (L1), and from the mesio-buccal gingival crevice of the left upper first molars (U6) and the left lower first molars (L6), at four different times: at baseline, before placement of orthodontic appliances (T1), and 1 week (T2), 3 months (T3), and 6 months after placement of orthodontic appliances (T4). All subjects received tooth brushing instructions, but no type of professional prophylaxis was performed during the observation periods.

Sampling sites were isolated with sterile cotton rolls and were dried by a gentle air stream. Then sterile paper points (DiaDent, Seoul, South Korea) were inserted about 1 mm into the gingival crevice and were left in situ for 30 seconds (Figure 1). These paper points were transferred immediately into Eppendorf tubes containing 250 mL distilled water and were kept in a freezer at −20°C maximum temperature.

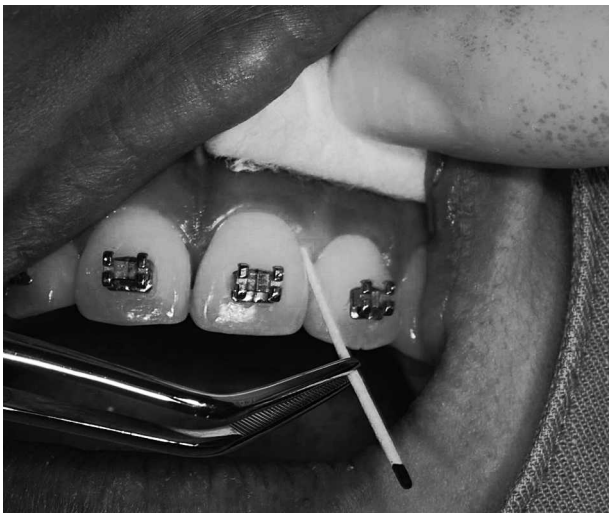

Figure 1. The procedure of subgingival plaque sampling.

DNA was extracted from the samples using the method of Mättö et al.[15] In this study, the 16S rRNA-based PCR detection method was used to determine the prevalence of *Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Campylobactor rectus, Eikenella corrodens, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens,* and *Treponema denticola,* which are considered putative periodontopathogens.[16,17] The PCR process of mixing a reagent, amplification, electrophoresis, staining, and visualization by ultraviolet transillumination has been described previously.[18]

Statistical Analysis

The McNemar test was used for pairwise comparisons of the frequency of periodontopathogens between periods. Logistic regression analysis of ordinal data was used to assess the effects of orthodontic appliances on bacterial colonization between sampling sites. The odds ratio and the 95% confidence interval for the change (X-O), representing microorganisms not existing at T1 but newly developing at T2, T3, and T4, were calculated. Because sites are categorical variables, they were treated to dummy variables, and U1 and L6 were set as reference sites. Differences were considered significant at P values less than .05. Data were processed with SAS, version 9.12 for Windows (SAS Institute Inc, Cary, NC) and the Statistical Package for the Social Sciences (SPSS), version 14.0 for Windows (SPSS Inc, Chicago, Ill).

RESULTS

Results are presented as the frequency (percentage) of sites positive for each species, and frequencies at T1, T2, T3, and T4 are summarized in Table 1. The frequency of subgingival plaque samples positive at T1 was 1.7% for *A. actinomycetemcomitans*, 7.5% for *T forsythia*, 44.2% for *C rectus*, 35.8% for *E corrodens*, 6.7% for *P gingivalis*, 5.0% for *P intermedia*, 25.8% for *P nigrescens*, and 11.7% for *T denticola* (Table 1).

The frequency of *C rectus* and *P nigrescens* at T2 was 65.0% and 44.2%, respectively, and differences in frequency between T1 and T2 were statistically significant ($P < .05$). The frequency of *T forsythia* was increased to 25.8% at T3 and to 36.7% at T4, and differences in frequency between T2 and T3 and between T3 and T4 were statistically significant ($P < .05$). The frequency of the other species also tended to increase after placement of orthodontic appliances, although it did not reach the level of statistical significance (Table 1 and Figure 2).

The frequency of X-O changes after placement of orthodontic appliances was higher at the upper first molars and the lower first molars than at the upper central incisors and the lower central incisors (Table 2 and Figure 3) and differed significantly between sites (Table 3).

DISCUSSION

This study evaluated changes in the microbiologic composition of subgingival plaque before and after the placement of fixed orthodontic appliances. The placement of orthodontic appliances and the leveling and alignment of crowding probably change the ecosystem of the mouth. The present study focused on the initial period of orthodontic treatment, that is, from the initial placement of the orthodontic appliances to the leveling and alignment period. Investigators also evaluated differences in frequency of X-O changes among the sampling sites.

The PCR method employed in this study is sufficiently sensitive and specific, especially for detecting anaerobic bacteria.[16] Mättö et al.[15] detected *P gingivalis* three times more frequently by PCR than by culture. 16S rRNA genes appear to be the most useful targets for PCR; they are present in every bacterium and are highly conserved within a single species.[19]

As shown in Table 1, *C rectus* (44.2%) and *E corrodens* (35.8%) were frequently detected preorthodontically at T1, whereas *A actinomycetemcomitans* (1.7%), *T forsythia* (7.5%), *P gingivalis* (6.7%), and *P intermedia* (5.0%) were scarcely detected. However, Umeda et al.[20] reported that in gingivally healthy children, the frequencies of *A actinomycetemcomitans*, *T forsythia*, *C rectus*, *P gingivalis*, *P nigrescens,* and *T denticola* were 1.8%, 42.9%, 94.6%, 8.9%, 42.9%, and 48.2%, respectively. The high frequency of periodontopathic bacteria in gingivally healthy subjects reported in that study is probably due to the use of different bacterial sampling techniques, including using curettes for subgingival plaque sampling, which removes more microorganisms than the paper tips used in this study.[21] However, using curettes for plaque sampling has a prophylactic effect, which has the potential to alter the findings at T2, T3, and T4. Therefore, this method is not appropriate for evaluating the effects of orthodontic treatment on subgingival microbiota.

Table 1 and Figure 2 show that orthodontic appliances promote the colonization of gingival crevices with periodontal pathogens. Our findings reject the null hypothesis and support previous reports showing that the composition of subgingival microbiota is affected by the placement of fixed orthodontic appliances, and that the prevalence of periodontopathogens increases over time.[1,3,5,10,13,22] One week after the placement of orthodontic appliances (T2), the frequencies of *C rectus* and *P nigrescens* were significantly elevated

Table 1. Frequency of Periodontopathogens in Subgingival Plaque at Baseline (T1) and at 1 Week (T2), 3 Months (T3), and 6 Months After Placement of Orthodontic Appliances (T4)

	T1		T2		T3		T4		*P* Value[a]			
	n	%	n	%	n	%	n	%	T1 vs T2	T2 vs T3	T2 vs T4	T3 vs T4
A actinomycetemcomitans												
Total (n = 120)	2	1.7	1	0.8	1	0.8	5	4.2				
U1 (n = 30)	1	3.3	0	0.0	0	0.0	0	0.0	NS	NS	NS	NS
U6 (n = 30)	0	0.0	0	0.0	0	0.0	0	0.0	NS	NS	NS	NS
L1 (n = 30)	1	3.3	0	0.0	0	0.0	2	6.7	NS	NS	NS	NS
L6 (n = 30)	0	0.0	1	3.3	1	3.3	3	10.0	NS	NS	NS	NS
T forsythia												
Total (n = 120)	9	7.5	7	5.8	31	25.8	44	36.7				
U1 (n = 30)	1	3.3	0	0.0	4	13.3	4	13.3	NS	NS	NS	NS
U6 (n = 30)	3	10.0	3	10.0	13	43.3	16	53.3	NS	.013*	.002**	NS
L1 (n = 30)	3	10.0	2	6.7	5	16.7	13	43.3	NS	NS	.003**	.021*
L6 (n = 30)	2	6.7	2	6.7	9	30.0	11	36.7	NS	.039*	.012*	NS
C rectus												
Total (n = 120)	53	44.2	78	65.0	78	65.0	83	69.2				
U1 (n = 30)	11	36.7	14	46.7	12	40.0	13	43.3	NS	NS	NS	NS
U6 (n = 30)	12	40.0	23	76.7	23	76.7	24	80.0	.007**	NS	NS	NS
L1 (n = 30)	17	56.7	21	70.0	21	70.0	23	76.7	NS	NS	NS	NS
L6 (n = 30)	13	43.3	20	66.7	22	73.3	23	76.7	NS	NS	NS	NS
E corrodens												
Total (n = 120)	43	35.8	52	43.3	50	41.7	50	41.7				
U1 (n = 30)	8	26.7	8	26.7	5	16.7	5	16.7	NS	NS	NS	NS
U6 (n = 30)	12	40.0	16	53.3	17	56.7	18	60.0	NS	NS	NS	NS
L1 (n = 30)	16	53.3	17	56.7	11	36.7	18	60.0	NS	NS	NS	NS
L6 (n = 30)	7	23.3	11	36.7	17	56.7	9	30.0	NS	NS	NS	NS
P gingivalis												
Total (n = 120)	8	6.7	7	5.8	12	10.0	15	12.5				
U1 (n = 30)	0	0.0	0	0.0	0	0.0	0	0.0	NS	NS	NS	NS
U6 (n = 30)	2	6.7	3	10.0	5	16.7	6	20.0	NS	NS	NS	NS
L1 (n = 30)	3	10.0	2	6.7	2	6.7	5	16.7	NS	NS	NS	NS
L6 (n = 30)	3	10.0	2	6.7	5	16.7	4	13.3	NS	NS	NS	NS
P intermedia												
Total (n = 120)	6	5.0	5	4.2	16	13.3	25	20.8				
U1 (n = 30)	1	3.3	0	0.0	3	10.0	5	16.7	NS	NS	NS	NS
U6 (n = 30)	1	3.3	2	6.7	5	16.7	7	23.3	NS	NS	NS	NS
L1 (n = 30)	3	10.0	0	0.0	4	13.3	7	23.3	NS	NS	NS	NS
L6 (n = 30)	1	3.3	3	10.0	4	13.3	6	20.0	NS	NS	NS	NS
P nigrescens												
Total (n =120)	31	25.8	53	44.2	51	42.5	62	51.7				
U1 (n = 30)	7	23.3	8	26.7	7	23.3	8	26.7	NS	NS	NS	NS
U6 (n = 30)	8	26.7	18	60.0	18	60.0	21	70.0	.013*	NS	NS	NS
L1 (n = 30)	11	36.7	13	43.3	14	46.7	17	56.7	NS	NS	NS	NS
L6 (n = 30)	5	16.7	14	46.7	12	40.0	16	53.3	.022*	NS	NS	NS
T denticola												
Total (n = 120)	14	11.7	8	6.7	18	15.0	17	14.2				
U1 (n = 30)	2	6.7	1	3.3	1	3.3	2	6.7	NS	NS	NS	NS
U6 (n = 30)	3	10.0	3	10.0	7	23.3	6	20.0	NS	NS	NS	NS
L1 (n = 30)	5	16.7	3	10.0	4	13.3	5	16.7	NS	NS	NS	NS
L6 (n = 30)	4	13.3	1	3.3	6	20.0	4	13.3	NS	NS	NS	NS

[a] McNemar test.
* $P < .05$; ** $P < .01$; NS, not significant.

from 44.2% to 65% and from 25.8% to 44.2%, respectively (Table 1). This indicates that these two strains may be more sensitive to environmental changes caused by the placement of orthodontic appliances than to the duration of orthodontic treatment. Ooshima et al.[23] suggest that *C rectus* and *P nigrescens* are common inhabitants of the normal oral flora in healthy children and play a limited role in the

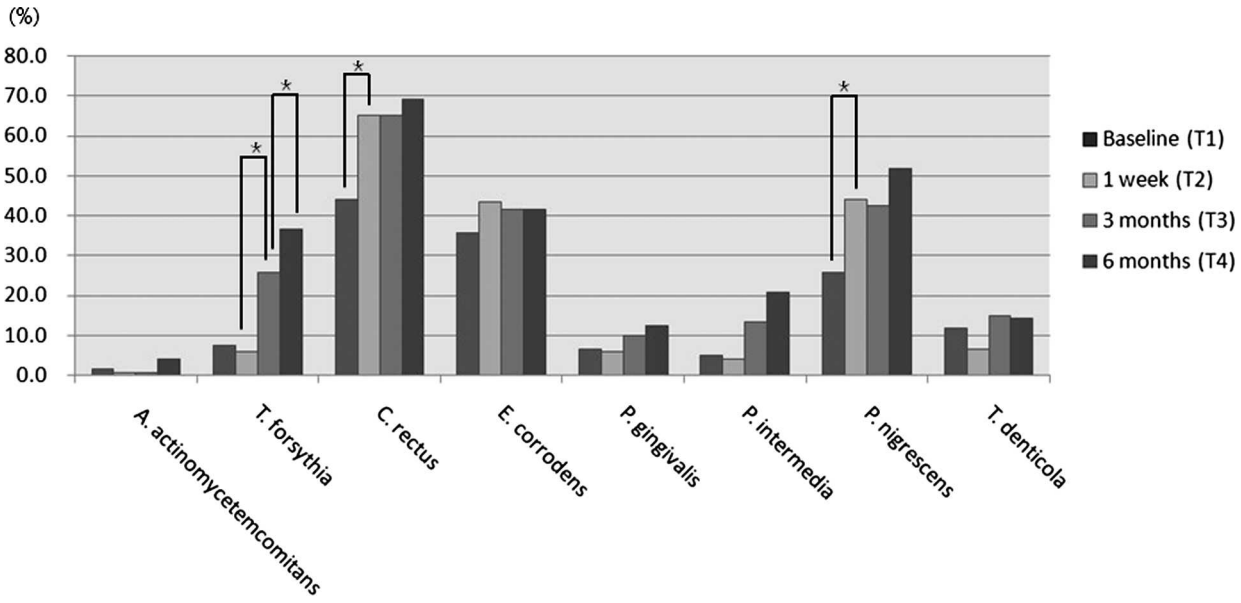

Figure 2. Percentage of sites positive for subgingival microorganisms at baseline (T1) and at 1 week (T2), 3 months (T3), and 6 months (T4) after placement of orthodontic appliances. * $P < .05$.

pathogenesis of periodontal disease. In recent studies on periodontally healthy children, the frequencies of *C rectus* and *P nigrescens* were greater than 80%; however, this may be due to familial transmission from the children's parents,[20,24] because many other studies report a high prevalence of these species in patients with gingivitis or periodontitis.[16,17,25] Ashimoto et al.[16] found the prevalence of *C rectus* and *P nigrescens* in subjects with advanced periodontitis to be 74% and 52%, respectively; further, Lee et al.[10] reported the prevalence of these species in subgingival plaque collected from gingivitis lesions in patients with fixed orthodontic appliances to be 88.2% and 47.3%, respectively. If these two strains are early indicators of periodontitis, the results of this study suggest that the progress of orthodontic treatment is closely related to periodontitis. Therefore, to prevent periodontitis, perfect oral hygiene education is a must after placement of orthodontic appliances.

The frequency of *T forsythia* did not change immediately after the placement of orthodontic appliances but increased significantly from 7.5% to 25.8% and 36.7% at 3 (T3) and 6 months (T4) after the placement of orthodontic appliances, respectively. This is higher than in the gingivitis subjects reported by Ashimoto et al.[16] (18%). Socransky et al.[17] categorized *T forsythia* into "red complex" species and *P nigrescens, and C rectus* into "orange complex" species according to their pathogenicity. They found *P nigrescens*, *C rectus,* and *T forsythia* to be closely related to pocket depth and bleeding upon probing, and suggested that although species within complexes are closely associated, the species in the orange complex group appear to be closely related to those in the red complex group.[17] The present study reconfirms the association between *P nigrescens*, *C rectus,* and *T forsythia.* Another interesting finding is that *C rectus* and *P nigrescens* appear to colonize immediately after the placement of orthodontic appliances, whereas *T forsythia* require a longer time to colonize. If the associations observed in this study are valid, *T forsythia* may be more effectively established in sites colonized by *C rectus* and *P nigrescens*, which may provide the nutritional requirements and/or binding sites for *T forsythia* by altering the physicochemical environment for microbial interaction.[26]

A. actinomycetemcomitans is associated with localized aggressive periodontitis.[12,13,20] Some authors have reported a very high frequency of *A actinomycetemcomitans* among orthodontic patients.[12,13] In the present study, however, *A actinomycetemcomitans* was detected at a very low level and showed no significant increase during the observation periods. Our finding was in accordance with the results of Demling et al.,[27] who did not find increased levels of *A actinomycetemcomitans* after insertion of fixed lingual appliances,

Table 2. Frequency of X-O Changes at All Sites[a]

	T1–T2		T1–T3		T1–T4	
	n	%	n	%	n	%
Upper incisor (U1)	15	6.3	22	9.2	25	10.4
Upper molar (U6)	42	17.5	60	25.0	67	27.9
Lower incisor (L1)	23	9.6	36	15.0	50	20.8
Lower molar (L6)	38	15.8	56	23.3	56	23.3

[a] X-O, None of the evaluated microorganisms existed at T1, but at least one species was detected at T2, T3, and T4.

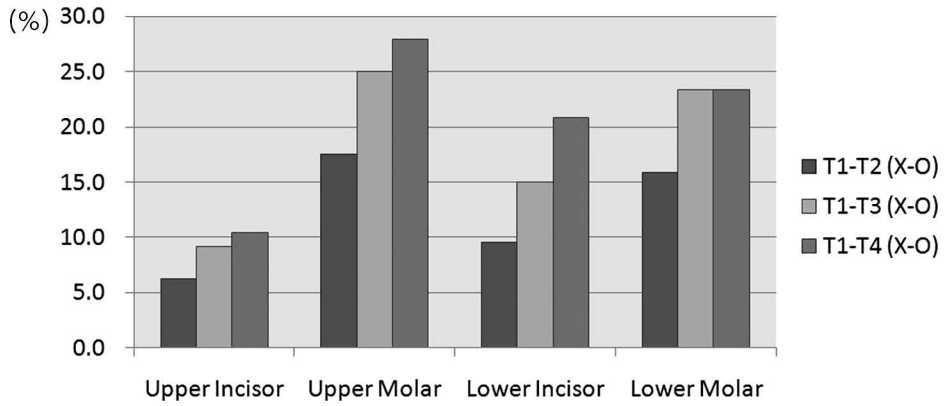

Figure 3. Percentage of X-O change, representing that microorganisms did not exist at T1 but were detected at T2, T3, and T4 at various sites.

although the clinical parameters were worse. This controversial finding might be caused by differences in the gingival health of subjects at baseline, in the host's individual susceptibility to *A actinomycetemcomitans*, and in the skill and effort of patients in maintaining oral hygiene during orthodontic treatment. From our short-term evaluation of the microbial changes, we may speculate that most orthodontic patients with a healthy gingival condition before the placement of orthodontic appliances would not experience severe destructive periodontitis.

It is known that gingival hyperplasia associated with brackets and bands is greater in the posterior than in the anterior teeth because the posterior teeth are fitted with bands, which often end subgingivally.[2,4,10] The reason for this is that preformed orthodontic bands invariably produce some overhangs that facilitate the accumulation of plaque and generate favorable conditions for the development of periodontopathogens.[2,5] Also in the present study, the frequency of sites with growth of the evaluated bacteria was higher at the molars than at the incisors (Table 1). We also evaluated the frequency of adverse change (X-O) representing PCR negativity at T1 but PCR positivity at T2, T3, and T4 between sites. X-O changes increased at all sites in a time-dependent manner (Table 2 and Figure 3) and were significantly greater at the molars than at the incisors (Table 3). This is probably correlated with difficulties during tooth brushing, or the fact that the incisors were bonded with brackets while the molars were fitted with bands.[5,22,28]

These results imply that complete plaque control is required immediately after the placement of fixed orthodontic appliances to maintain gingival health during orthodontic treatment—especially in teeth with bands. Whether the risk of periodontitis can be reduced by preparing more minute bands, which are strictly placed supragingivally, and/or by providing chemical or photodynamic plaque control with, for example, chlorhexidine has not been investigated yet.

Some studies have found that overall gingival changes produced by orthodontic appliances are transient with no permanent damage to periodontal tissues.[2,6,7] Our previous study also found that periodontopathogens present during orthodontic treatment were significantly reduced within 3 months after appliance removal.[18] However, the increase in the prevalence of periodontopathogens after placement of orthodontic appliances carries a risk of periodontal deterioration during 2 or 3 years of orthodontic treatment. This makes it even more important to provide professional prophylaxis if individual oral hygiene efforts are suboptimal. However,

Table 3. Odds Ratio of the X-O Change Between Sites[a]

	T1–T2		T1–T3		T1–T4	
Site	Odds Ratio	*P* Value[b]	Odds Ratio	*P* Value[b]	Odds Ratio	*P* Value[b]
Reference Site, U1						
U6	−0.065	.000***	−0.008	.503	0.031	.018*
L1	−0.013	.306	−0.015	.168	−0.011	.415
L6	−0.049	.000***	−0.009	.447	0.034	.015*
Reference Site, L6						
U6	−0.016	.254	0.001	.941	−0.003	.838
L1	0.049	.001**	0.009	.652	−0.034	.003**

[a] X-O, None of the evaluated microorganisms existed at T1, but at least one species was detected at T2, T3, and T4.
[b] Statistically significant differences between sites were tested using logistic analyses of ordinal data.
* $P < .05$, ** $P < .01$, *** $P < .001$.

additional studies are needed on the most effective way of controlling periodontopathogenic anaerobic bacteria during orthodontic treatment, especially in the molar region.

Periodontopathic anaerobic bacteria show a negative association with Gram-positive species and a positive association with Gram-negative species.[17,26] Consequently, the high prevalence of periodontopathic anaerobic bacteria and the low prevalence of Gram-positive species among orthodontic patients probably lead to increased periodontal risk because of the absence of antagonistic species.[26] Furthermore, the increase in the mobility of teeth and the hyperactivity of osteoclasts during orthodontic treatment probably aggravate the destructive periodontitis initiated by periodontopathogens.

CONCLUSIONS

- During orthodontic leveling and alignment, the frequency of *Tannerella forsythia, Campylobactor rectus,* and *Prevotella nigrescens* in gingival crevices increase significantly, thereby heightening the risk of periodontitis.
- Obviously, the convention oral hygiene measures performed by patients in this study were not able to overcome the development of a more periodontopathogenic flora in the gingival sulcus.

REFERENCES

1. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. *Am J Orthod Dentofacial Orthop*. 1991;100:337–340.
2. Kloehn JS, Pfeifer JS. The effect of orthodontic treatment on the periodontium. *Angle Orthod*. 1974;44:127–134.
3. Zachrisson BU. Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am J Orthod*. 1976;69:285–300.
4. Zachrisson S, Zachrisson BU. Gingival condition associated with orthodontic treatment. *Angle Orthod*. 1972;42:26–34.
5. Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances: a review. *J Periodontol*. 1996;67:78–85.
6. Polson AM, Subtelny JD, Meitner SW, et al. Long-term periodontal status after orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 1988;93:51–58.
7. Sadowsky C, BeGole EA. Long-term effects of orthodontic treatment on periodontal health. *Am J Orthod*. 1981;80: 156–172.
8. Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. *J Periodontol*. 1965;36:177–187.
9. Diamanti-Kipioti A, Gusberti FA, Lang NP. Clinical and microbiological effects of fixed orthodontic appliances. *J Clin Periodontol*. 1987;14:326–333.
10. Lee SM, Yoo SY, Kim HS, et al. Prevalence of putative periodontopathogens in subgingival dental plaques from gingivitis lesions in Korean orthodontic patients. *J Microbiol*. 2005;43:260–265.
11. Naranjo AA, Triviño ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. *Am J Orthod Dentofacial Orthop*. 2006;130:275.e17–e22.
12. Paolantonio M, di Girolamo G, Pedrazzoli V, et al. Occurrence of *Actinobacillus actinomycetemcomitans* in patients wearing orthodontic appliances: a cross-sectional study. *J Clin Periodontol*. 1996;23:112–118.
13. Paolantonio M, Festa F, di Placido G, D'Attilio M, Catamo G, Piccolomini R. Site-specific subgingival colonization by *Actinobacillus actinomycetemcomitans* in orthodontic patients. *Am J Orthod Dentofacial Orthop*. 1999;115:423–428.
14. Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol*. 1967;38(suppl):610–616.
15. Mättö J, Saarela M, Alaluusua S, Oja V, Jousimies-Somer H, Asikainen S. Detection of *Porphyromonas gingivalis* from saliva by PCR by using a simple sample-processing method. *J Clin Microbiol*. 1998;36:157–160.
16. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol*. 1996;11:266–273.
17. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25:134–144.
18. Choi DS, Cha BK, Jost-Brinkmann PG, et al. Microbiologic changes in subgingival plaque after removal of fixed orthodontic appliances. *Angle Orthod*. 2009;79:1149–1155.
19. Schmidt TM, Relman DA. Phylogenetic identification of uncultured pathogens using ribosomal RNA sequences. *Methods Enzymol*. 1994;235:205–222.
20. Umeda M, Miwa Z, Takeuchi Y, et al. The distribution of periodontopathic bacteria among Japanese children and their parents. *J Periodontal Res*. 2004;39:398–404.
21. Tanner AC, Goodson JM. Sampling of microorganisms associated with periodontal disease. *Oral Microbiol Immunol*. 1986;1:15–20.
22. Boyd RL, Baumrind S. Periodontal considerations in the use of bonds or bands on molars in adolescents and adults. *Angle Orthod*. 1992;62:117–126.
23. Ooshima T, Nishiyama N, Hou B, et al. Occurrence of periodontal bacteria in healthy children: a 2-year longitudinal study. *Community Dent Oral Epidemiol*. 2003;31:417–425.
24. Kulekci G, Leblebicioglu B, Keskin F, Ciftci S, Badur S. Salivary detection of periodontopathic bacteria in periodontally healthy children. *Anaerobe*. 2008;14:49–54.
25. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. *Periodontol 2000*. 1997;14:12–32.
26. Socransky SS, Haffajee AD, Dzink JL, Hillman JD. Associations between microbial species in subgingival plaque samples. *Oral Microbiol Immunol*. 1988;3:1–7.
27. Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W. Influence of lingual orthodontic therapy on microbial parameters and periodontal status in adults. *Eur J Orthod*. 2009;31:638–642.
28. Miethke RR, Bernimoulin JP. Effects of bands and brackets on the marginal periodontium. *Fortschr Kieferorthop*. 1988; 49:160–169.