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ORIGINAL ARTICLE

Changes in the supragingival microbiota surrounding brackets of upper central incisors during orthodontic treatment

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Abstract

Objective. The aim of this study was to determine how fixed orthodontic appliances affect microbiota of supragingival plaque over 5 months. ***Materials and methods.*** Twenty individuals of Scandinavian origin, aged 10–16 years, were included. All subjects were fitted with fixed orthodontic appliances in both the maxillary and mandibular tooth arches. Pooled supragingival plaque samples from the labial surface of the two maxillary central incisors were collected before bonding (T1) and afterwards at 4 weeks (T2), 3 months (T3) and 5 months (T4). The plaque index (PI) was recorded for each sampling. The gingival status was documented at T1 and T4 by using clinical photographs. Plaque microbiota was identified using the Human Oral Microbe Identification Microarray (HOMIM). ***Results.*** Increased plaque levels were recorded after bonding, however the increase was not significant. The prevalence of gingivitis at the maxillary central incisors increased from 25% at T1 to 74% at T4. No significant changes of the plaque microbiota from the sample area were detected during the 5-month period. Trends toward a microbiota containing more periodontitis- and caries-associated bacteria were detected. ***Conclusions.*** Although trends toward a microbiota containing more periodontitis- and caries-associated bacteria were detected, the changes were not severe enough to be significant. Treatment with fixed orthodontics does not necessarily shift the microbiota to a more pathogenic composition.

Key Words: *fixed orthodontics, HOMIM, molecular microbiology, supragingival plaque*

Introduction

Fixed orthodontic appliances create many retention sites [1] on tooth surfaces that normally contain low levels of plaque, such as labial and buccal smooth surfaces. The plaque composition at these retention sites differs in both chemistry and microbiology compared to before placement of the appliances. In addition to lower pH, higher levels of calcium, phosphate and carbohydrate levels [2], the plaque contains an increased number of species associated with caries [3,4] and periodontitis [4,5]. It has been shown that dental conditions such as white spot lesions (WSLs) [6–8], gingivitis [5,9] and halitosis [9] also frequently develop in patients treated with fixed orthodontic appliances. Orthodontists routinely give their patients oral health education and instruction on plaque-control techniques to minimize the occurrence of plaque. Even orthodontic patients with good oral hygiene show marked changes of the oral microbiota [10,11].

Previous studies examining modification of the oral microbiota in patients treated with fixed orthodontics used cultivation-based identification approaches. As ~ 35% of microbial species out of at least 600 predominant oral microbes remains uncultivated [12], it is likely that our understanding of the microbial changes related to orthodontic treatment is incomplete. In recent years, molecular identification techniques have provided a better understanding of the microbial profiles associated with oral health [13], caries [14], white spot lesions [13] and periodontitis [15].

The aim of this study was to determine how the supragingival plaque microbiota changes during the first 5 months of fixed orthodontic treatment and to see how clinical parameters such as plaque index and gingival status are related to these changes. To

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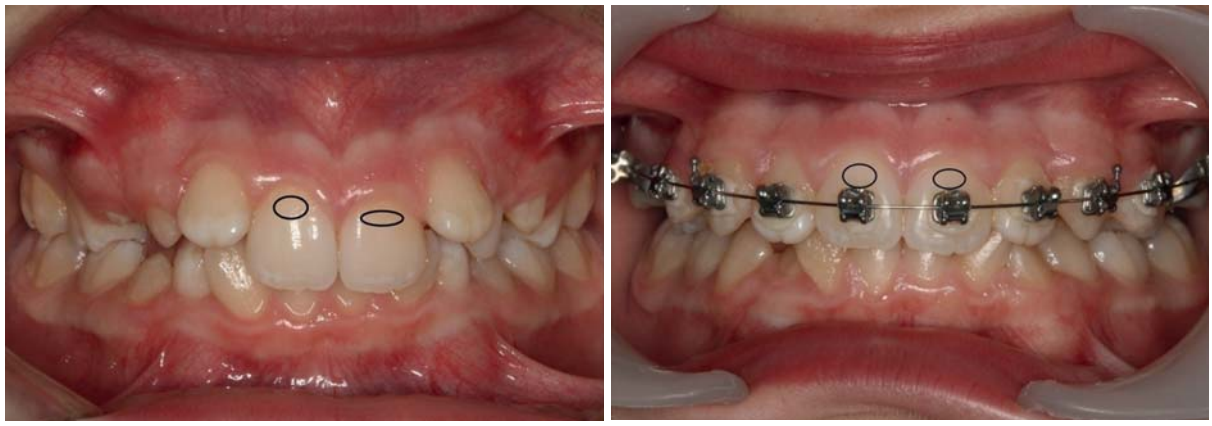


Figure 1. The sampling area before and after bonding.

identify the oral microbiota, we used a 16S rRNA-based microarray method, the Human Oral Microbe Identification Microarray (HOMIM) [15], a high-throughput method that allows simultaneous detection of ~ 300 of the most prevalent oral bacterial species, including those that cannot yet be cultivated [http://mim.forsyth.org/].

Materials and methods

Subject population

The study was approved by the Regional Ethics Committee (REK sør-øst, Blindern, Oslo, Norway). All patients and their guardians signed a consent form before participating in this study. The subject population consisted of 20 individuals of Scandinavian origin, eight of whom were male. The subjects were between 10–16 years old (mean age = 12, SD = 1 year), undergoing treatment with fixed orthodontics in both maxilla and mandibula. The bonding protocol was the same for all patients; 37% phosphoric acid, primer and adhesive from Rely-a-Bond® (Reliance Orthodontic Products, Inc., Itasca, IL). All patients were treated with metal brackets, using one of the following fixed orthodontic apparatuses; 3M Victory brackets (3M Corporate Headquarters, St. Paul, MN), Ormco Orthos Titan (http://www.ormco.com/index/ormco-products-advansync), Ormco Damon (http://www.ormco.com/index/ormco) or GAC In-ovation (http://www.gacinovation.com/).

The patients did not brush their teeth the morning prior to sample taking and had nothing to eat within an hour before sampling. However, patients were instructed to continue with their regular oral hygiene regime for the duration of the study. If poor oral hygiene was detected at any visit, the orthodontic team at the clinic informed and re-motivated the patient to improve oral hygiene habits. All appointments took place between 8 am and 12 pm (noon). Since supragingival plaque samples were taken from the same patients before an orthodontic appliance was inserted, the patients served as their own controls (T1).

The maxillary central incisors were chosen for this study since the concentration of fluoride, that prevents dental caries, has been shown to be lowest in plaque on the upper front teeth [16].

Clinical procedures

A lip spreader was used to isolate the area around the two maxillary central incisors. The buccal surfaces of the incisors were then air-dried. Care was taken not to touch the sample areas. Supragingival plaque samples were collected using a sterile Gracey curette from a defined location on the buccal tooth surface near the bracket (Figure 1). The samples were collected at four times: T1, less than an hour prior to bonding; T2, 4 weeks after bonding; T3, 3 months after bonding; T4, 5 months after bonding (Table I). Standardized clinical photographs were taken of the sampling area at T1 and T4. These photographs were used to visually evaluate and longitudinally compare the clinical condition of the gingiva in proximity to the sample area. Visual signs of gingivitis were recorded (Figure 2). The plaque index was recorded during each sampling: no clinically visible plaque after running a curette over the enamel surface = 0, light plaque after running a curette over the enamel surface = 1 and heavy plaque = 2.

The plaque samples were immediately suspended in 300 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) in a 2 ml micro tube (Sarsdent, Art. No. 72.694.006), transported to the laboratory using the Nalgene™ Labtop Cooler (CAT. NO. 5115-0032 [−20°C]), and stored at −80°C until analyzed. All samples were collected by the same examiner (LT).

DNA isolation and HOMIM protocol

DNA was isolated from clinical samples using a Ready-Lyse™ Lysozyme Solution (Epicentre, Madison, WI) for overnight incubation before applying a

Table I. Time interval between visits.

	Number of patients	Mean number of weeks	SD (weeks)
T1 to T2	20	4.20	0.951
T2 to T3	19	8.21	0.855
T3 to T4	18	7.22	1.396

T1 – less than an hour prior to bonding, T2 – four weeks after bonding, T3 – three months after bonding, T4 – five months after bonding

MasterPure DNA Purification Kit (Epicentre) according to the manufacturer's directions. Purified DNA samples were analyzed using HOMIM. The method is described in detail elsewhere [15]. Briefly, 16S rRNA-based, reverse-capture oligonucleotide probes (typically 18–20 bases) were printed on aldehyde-coated glass slides. 16S rRNA genes were PCR amplified from DNA extracts using 16S rRNA universal forward and reverse primers and labeled via incorporation of Cy3-dCTP in a second nested PCR. The labeled 16S rRNA gene amplicons were hybridized overnight with probes on the slides. After washing, the microarray slides were scanned using an Axon 4000B scanner and crude data were extracted using GenePix Pro software (Molecular Devices, LLC, Sunnyvale, CA).

Analysis of microbial community profiles

The analysis of the HOMIM microarrays was performed by first scanning the arrays to make a digital image on the computer. Then a HOMIM online analysis tool (available at: http://bioinformatics.forsyth.org/homim/) was used to interpret these images and create microbial community profiles. Each green fluorescent spot on the array represented a unique probe for a particular taxon. The fluorescent spots varied in intensity based on how much of the target DNA was present and therefore on the level of hybridization. We calculated a mean intensity for each spot (taxon) of the same probe and the signals were normalized and calculated as previously described [15]. The signal intensities for each taxon were then used to calculate the signal range by raising them to the power of 0.3. To minimize background noise all original signals that had intensities less than twice the background value were reset to 1 and assigned to the signal level 0 (meaning that that particular taxon was absent in the sample). The original signals that had values over 1 were re-categorized into scores from 1–5. These results were then used to compare the community composition variation between samples using correspondence analysis (CoA) in MeV 4.6 [17]. Analysis was performed on the absolute intensity of the HOMIM data (frequency of scores from 0–5) as well as binary data (presence/absence). Only T1, T2 and T4 samples were used in the HOMIM analysis. T3 was excluded due to a large variation of the sampling date. The prevalence of each taxon was computed for each subject and averaged within groups. Wilcoxon Rank Sum test and *t*-test were used to identify statistically significant differences (T1 and T4 and plaque indices 0, 1 and 2). A p-value of < 0.05 was considered significant. False discovery rate (FDR) using Benjamini-Hochberg correction [13] was used to control for multiple hypotheses.

Results

Clinical parameters

The percentage of patients who had a heavy plaque accumulation covering the sample area increased from 10% to 30% after bonding of the fixed orthodontic appliance (Figure 3), however this increase was not significant: T1 with T2 (Fisher's Exact Test: p-value = 0.06), T1 with T4 (Fisher's Exact Test: p-value = 0.16). None of the subjects developed clinically visible WSLs on the sampling area during the observation period. The presence of gingivitis around the maxillary central incisors increased significantly

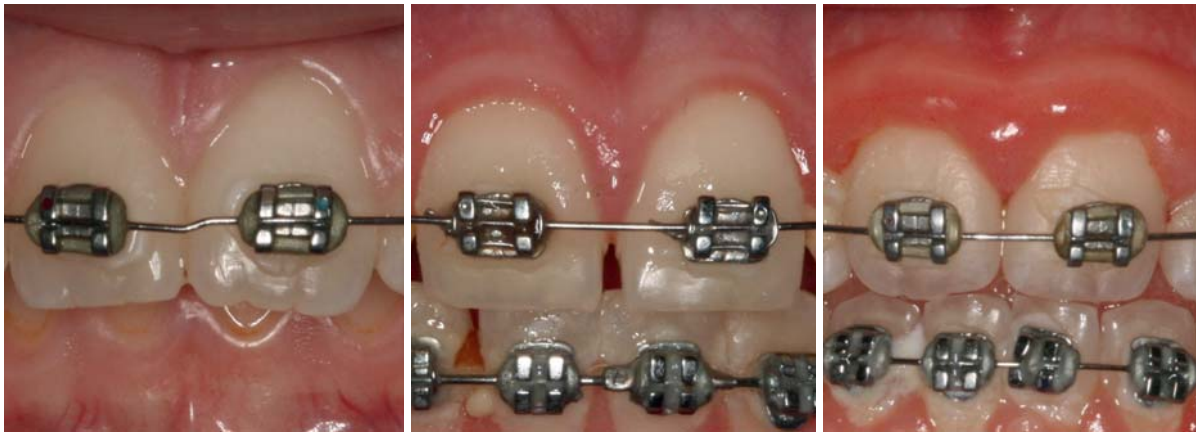
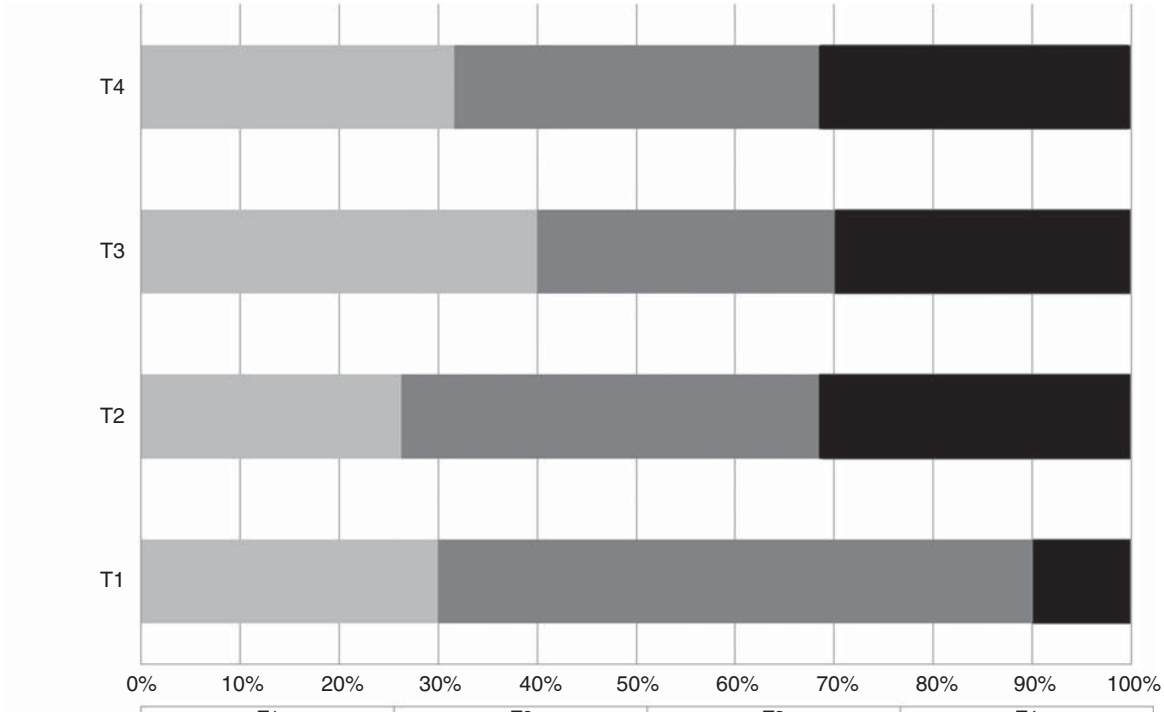


Figure 2. Visual assessment of gingival status.



	T1	T2	T3	T4
No plaque	30	26.3	40	31.6
Light plaque	60	42.1	30	36.8
Heavy plaque	10	31.6	30	31.6

Figure 3. Plaque levels covering sample area for each time period.

from 25% at T1 to 74% at T4 (Fisher's Exact Test: p-value = 0.03) (Figure 4). There was no significant connection between the plaque level from our sample area and the observed gingivitis (Fisher's Exact Test: T1 p-value = 0.21 and T4 p-value = 0.40), meaning that the level of gingival inflammation was not dependent on the plaque levels adjacent to the brackets.

Microbial community shift induced by treatment with fixed orthodontics

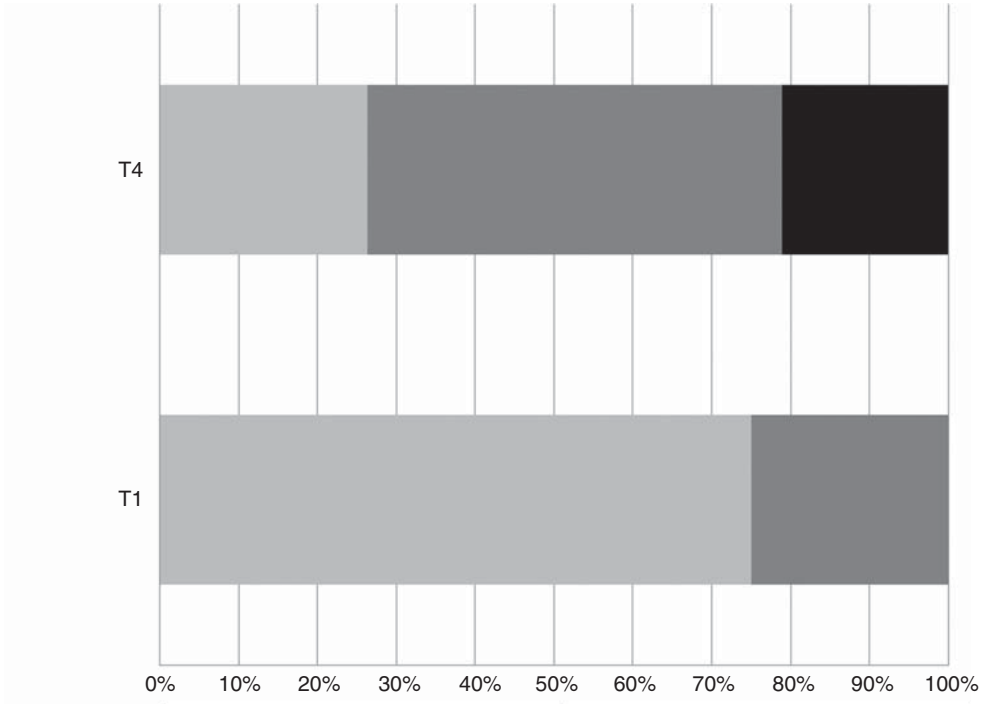
Clustering analysis did not show any clear grouping for periods T1, T2 and T4 (Figure 5). From the figure, we can also see that the microbial profiles were similar over time. However, certain trends were detected, especially when comparing T1 to T4 (Figure 6); after 5 months of treatment with fixed orthodontics, the percentages of 13 bacterial species were elevated while 21 were reduced in number. The microbial community surrounding the orthodontic brackets had become more specific, containing elevated levels of species associated with periodontitis, e.g. *Tannerella forsythia*, *Campylobacter concisus* and/or *C. rectus*, and a few bacterial species associated with white spot lesions such as *Acidaminoccaceae* sp. OT155, *Lactobacillus* spp., *Solobacterium moorei* and *Eubacterium* sp. OT082 [13]. There was a decrease in species associated with dental health, e.g. *Streptococcus anginosus*, *S. intermedius* and *S. oralis*. None of these changes were significant when the p-values were corrected for FDR.

Microbial community with respect to plaque index

Figure 7 shows that a population of 32 bacterial species was increased and seven were decreased in heavy plaque (PI = 2) samples as compared to samples that had no clinically visible plaque (PI = 0). Bacterial species associated with caries such as *Lactobacillus* spp. and bacteria associated with periodontitis, i.e. *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *C. concisus* and *C. rectus*, were elevated in PI = 2 samples. Bacterial species associated with dental health such as *S. oralis*, *S. sanguinis* and *Streptococcus* Cluster I were decreased. Again these changes were not significant when the p-values were corrected for FDR.

Microbial community with respect to visual assessment of the gingival status

The microbial community was also analyzed with respect to gingival status. Four sample pairs had to be excluded from the analyses as they did not have their respective pair at T1 or T4. The remaining sample size was very small and no significant differences in the microbiota were detected.



	T1	T4
Healthy gingiva	75%	26.30%
Mild gingivitis	25%	52.60%
Severe gingivitis	0%	21.10%

Figure 4. Gingival status for patients at T1 and T4. 0: healthy gingiva. 1: mild gingivitis. 2: severe gingivitis.

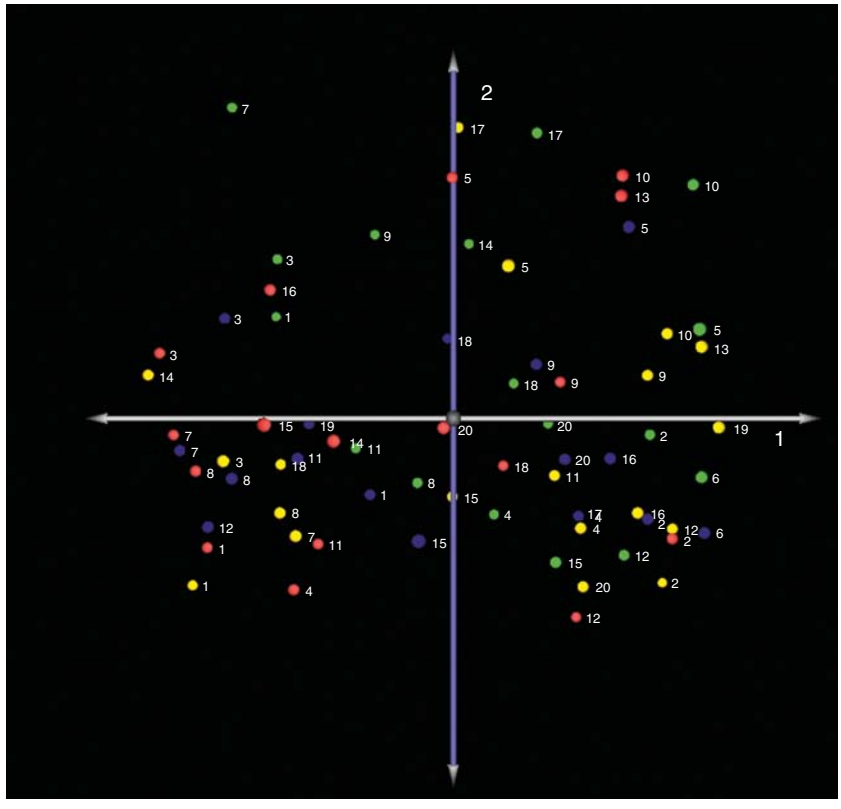


Figure 5. PCA analysis. Showing the relationship between the microbial composition of samples. Green – T1, Blue – T2, Orange – T3, Red – T4. Numbers are patient samples.

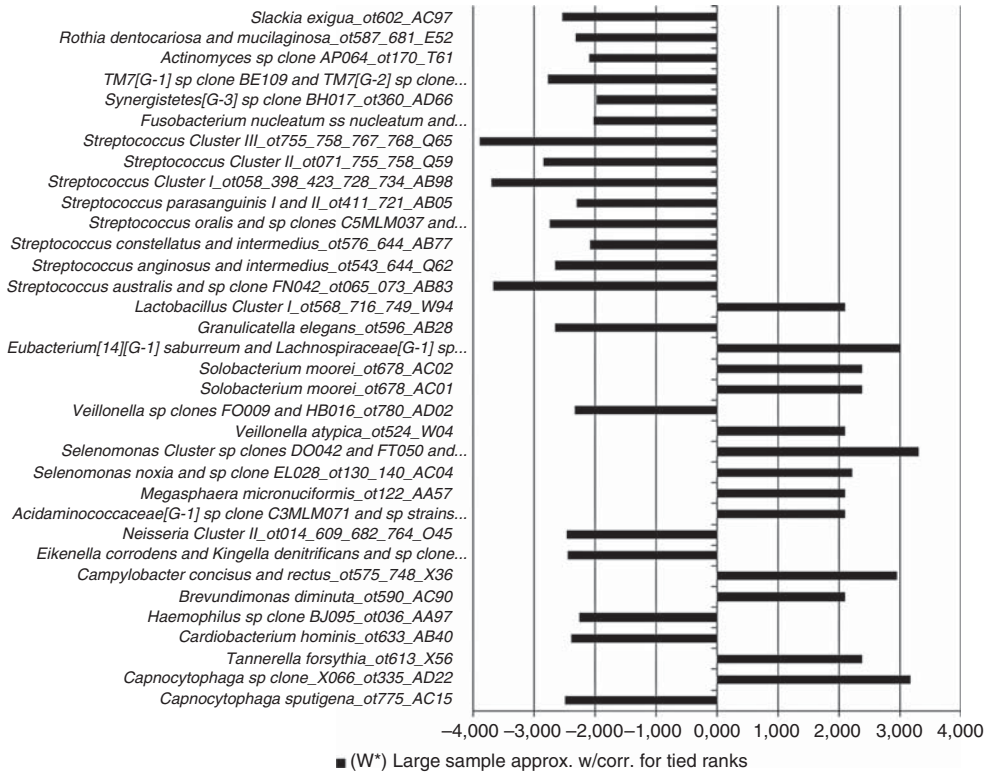


Figure 6. Changes in the microbial community from T1 to T4.

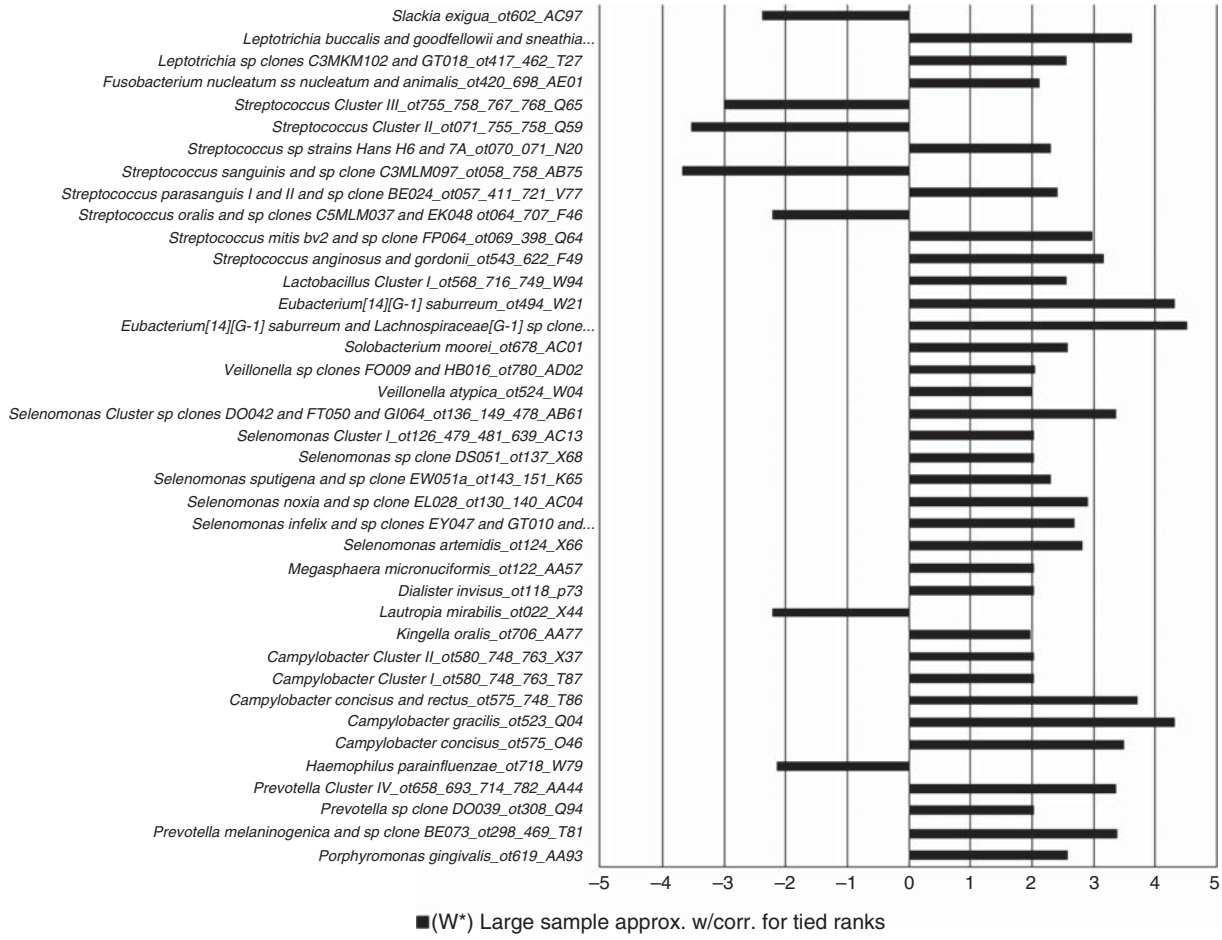


Figure 7. Differences in microbial community composition between PI 0 and PI 2 groups.

Discussion

Many studies have shown that the bacterial composition changes significantly during fixed orthodontic treatment [3–5]. Our aim was to gain a better understanding of these changes by using a molecular method that allows simultaneous detection of ~ 300 of the most prevalent oral bacterial species. It was, therefore, surprising to find only trends, but no significant changes in the microbiota. There are several possible reasons for this; (1) our patient group did not have a significant increase of plaque levels from the sampling site after bonding and (2) our sampling site differed from previous studies.

Even though no significant changes were detected, this study provides an overview of the bacterial composition found surrounding orthodontic brackets on upper central incisors. Clear trends were seen (Figure 6) after bonding of the brackets, showing a decrease of bacterial species associated with dental health (including *S. anginosus*, *S. intermedius* and *S. oralis*), an increase of pathogens assosiated with dental disease (including *T. forsythia*, *S. moorei*, *Capnocytophaga* spp. and *Lactobacillus*) and an increase of a few bacterial species associated with white spot lesions. This is in agreement with previous studies that found that placement of fixed orthodontic appliances was associated with an increase in putative periodontal pathogens in subgingival plaque [4,18]. It is not surprising to find an increase in species associated with periodontitis after bracket placement, since gingivitis is common during orthodontic treatment [5,9] and it has been shown that species associated with periodontitis are commonly found in sites of gingival inflammation, even with minimal attachment loss and pocket depths [19]. However, in the present study, we observed that periodontal pathogens increased in supragingival plaque. This finding suggests that supragingival and subgingival plaque are not completely isolated microbial habitats and that the microbial composition of one may affect the other [20].

It is well known that plaque levels generally increase during treatment with fixed orthodontics. However in our subject population we did not find a significant increase in plaque levels during the 6-month study duration surrounding the upper central incisor brackets. This may be due to the fact that upper incisors are easily accessible for oral hygiene or the subjects were extra meticulous in their oral hygiene habits because they were participating in the study. Nevertheless, clear trends were seen when comparing sample areas with no visible plaque and those with heavy plaque (Figure 7). The population of several bacterial species associated with dental disease increased (*P. gingivalis*, *C. concisus* and *C. rectus*, *S. moorei* and lactobacilli).

White spot lesion development during fixed orthodontic treatment is a common clinical problem. Although none of the subjects developed clinical WSLs during the observation period, the placement of the fixed appliances led to an increase in some of the bacterial species often found to be associated with WSL development [13]. However, our study did not find an increase in *Streptococcus mutans* as some studies have reported [3,13,21]. These latter studies sampled plaque adjacent to orthodontic bands, while our study sampled plaque from teeth adjacent to orthodontic brackets. It has previously been shown that *S. mutans* levels are higher in plaque adjacent to orthodontic bands as compared to metal brackets [22].

This study showed that at the maxillary central incisors, treatment with fixed orthodontic appliances does not necessarily cause a significant increase in plaque levels or a significant shift in the composition of plaque microbiota, even though a significant increase of gingivitis was recorded.

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