Salivary microbiome and periodontal status of patients with periodontitis during the initial stage of orthodontic treatment

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Introduction: Patients with severe periodontitis typically present with pathologic tooth migration. To improve esthetics and masticatory function, orthodontic treatment is required. Research on periodontal orthodontic treatment has been sparse, particularly from the microbial perspective. Hence, we analyzed the microbial and clinical changes in patients with well-controlled periodontitis in the early stage of orthodontic treatment. **Methods:** Ten patients with well-controlled periodontitis were asked to collect saliva before and 1 and 3 months after appliance placement (T0, T1, and T2, respectively) and underwent clinical examinations before and 1, 3, and 6 months after appliance placement (T0, T1, T2, and T3, respectively). The microbial community of saliva was analyzed by 16S rRNA gene sequencing. Gingival index, the plaque index, and the probing pocket depth were clinically assessed. **Results:** The plaque index significantly increased from T0 to T1 and decreased at T2 and T3. The probing pocket depth and gingival index increased slightly at T2, but not significantly, in both the high-risk site and low-risk site. The alpha and beta diversity increased at T1. The microbial community structure was similar at T0 and T2. The relative abundance of core genera and periodontal pathogens was stable during the initial 3 months of orthodontic treatment. **Conclusions:** The orthodontic appliance promoted plaque accumulation and altered the microbial community of patients with well-controlled periodontitis during the first month of orthodontic treatment. The microbial community returned to the basal composition at 3 months after appliance placement, and the periodontal inflammation during the 6-months orthodontic treatment was under control. (Am J Orthod Dentofacial Orthop 2021;159:644-52)

Periodontitis is a common disease among adults, and its incidence increases with age. Patients with severe periodontitis typically present with occlusal interference, irregular spacing, proclined incisor, deepbite, and deep jet.[1] These pathologic changes in tooth position necessitate combined periodontal orthodontic treatment. Severe periodontitis is no longer considered a contraindication for orthodontic treatment.[2-4] With adequate plaque control and proper force application, a tooth with decreased support by the alveolar bone can undergo orthodontic treatment without jeopardizing periodontal health.[4] Most clinical studies have shown no periodontal tissue destruction after placement of an orthodontic appliance.[2,5,6]

Periodontitis has high rates of progression and relapse.[7] The stagnant area around the fixed appliance is difficult to clean, and new occlusal interference might occur during the tooth movement. Therefore, patients with severe periodontitis receiving orthodontic treatment are at risk of the relapse of periodontal inflammation. If inflammation is not fully controlled during orthodontic treatment, tooth movement can cause additional attachment loss. Hence, efficient periodontal screening should be performed during orthodontic treatment because clinical and radiographic examinations have limited ability to evaluate the risk for periodontitis.

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Microbial infection plays an important role in the mechanism of periodontitis. Changes in the microbiome of subgingival plaque precede clinical signs of periodontitis, such as gingival swelling, attachment loss, deep pockets, and bone loss. The salivary microbiota is positively correlated with subgingival microbiota[8,9] Indeed, the presence of periodontal pathogens in the subgingival plaque can be reflected in saliva.[10] Thus, because saliva sampling is noninvasive and convenient, changes in the microbial composition of saliva could be an indicator of periodontal health.

Our previous systematic review indicated that the relative abundance of periodontal pathogens varies in patients over time.[11] In addition, 16S rRNA gene sequencing has shown that disruption of the structure and composition of the microbiome is associated with periodontal inflammation.[12] In this study, we evaluated the composition and diversity of the microbiome. We also analyzed the microbial and clinical parameters of patients with well-controlled periodontitis in the early stage of orthodontic treatment.

MATERIAL AND METHODS

Ten patients with well-controlled periodontitis (6 women and 4 men; age range, 30-45 years; mean age, 36.4 years; standard deviation [SD], 2.1) were analyzed. After completing periodontal treatment, the 10 patients were referred by their periodontist for orthodontic treatment. The ethics committee approved the study protocol, and all of the patients provided written informed consent. Patients with the following conditions were included in this study: aged 30-50 years; periodontitis stage III or IV and grade C (attachment loss of 30% of teeth of more than 5 mm, and radiographs showing alveolar bone loss around at least half the length of the root[13]); systematic periodontal treatment within 3 months and a stable periodontal condition (no pocket with >4 mm probing pocket depth [PPD], plaque index [PLI] <30%, gingival index [GI] <30%, and no occlusal trauma); mild tooth crowding or spacing; good oral hygiene behavior and no smoking; no crowns, implants, or fixed bridges; no diabetes or other systemic disease; no pregnancy; and had not taken antibiotics or hormones within 3 months.

All of the patients underwent fixed appliance treatment for both maxillary and mandibular arch at the fourth-week visit. Metal brackets (Shinye, Hangzhou, China), a nickel-titanium archwire (Shinye), and stainless steel archwires were used for orthodontic treatment. Bands and excess cement residues were avoided. Light force (50-100 g of force) was applied. During the first 6 months of treatment, oral hygiene instructions, including modified bass brushing and flossing using an interdental brush, were given for each including patient at each visit. Periodontal treatments and antibacterial mouthwashes were prohibited.

The patients provided saliva before appliance placement (T0) and 1 (T1) and 3 (T2) months after appliance placement and underwent clinical examinations at T0, T1, T2, and 6 months after appliance placement (T3). Saliva sampling was performed before the clinical examination. Unstimulated saliva samples were collected in the morning, and the patients were asked to avoid eating and brushing their teeth for at least 8 hours. The patients were instructed to pool the saliva into a sterile tube for 5 minutes. Saliva samples were stored at −80°C.

After saliva sampling, GI, PLI, and PPD were clinically assessed. The GI was assessed using the following classification: (0) healthy gingiva, no bleeding; (1) edema, change in color without bleeding; (2) bleeding without flow along the gingival margin; (3) bleeding with the flow along the gingival margin; (4) copious bleeding; and (5) severe inflammation with a tendency to spontaneous bleeding. The PLI was assessed using the following classification: (0) no plaque, (1) flecks of plaque at the gingival margin, (2) definite line of plaque at the gingival margin, (3) plaque covering less than one-third of the tooth surface, (4) plaque covering more than one-third and less than two-thirds of the tooth surface, and (5) plaque covering more than two-thirds of the tooth surface. Six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) of each tooth were recorded by an experienced periodontist. First, the overall periodontal status is presented as the full mouth means on the basis of the 6 measurements of each tooth. Second, the sites were divided into 2 subgroups: high-risk site (attachment loss ≥5 mm at T0) and low-risk site (attachment loss <5 mm at T0). PPD and GI were also assessed in 2 subgroups. According to periodontal risk assessment, the suggested recall interval is 3 months. Hence, the PPD and GI were assessed every 3 months and were not evaluated at T1.

Genomic DNA was isolated from saliva samples using Tiangen Bacteria DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The DNA concentration was measured using the NanoDrop ND1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Mass). The v3-v4 regions of the bacterial 16S rDNA gene were amplified by polymerase chain reaction, and the products were deep sequenced on the MiSeq Platform at the Auwigene Institute (Beijing, China). Next, image analysis, base calling, and error estimation were performed using Illumina Sequencing Analysis Pipeline (version 2.6; Genome Analyzer, Illumina, Inc, San Diego, Calif).[14] The sequence data obtained in our study have been deposited in the

National Center for Biotechnology Information Sequence Read Archive database under Accession No. SUB5553353.

The Mothur software package and the Quantitative Insights Into Microbial Ecology pipeline were used to analyze the sequencing data further. Low-quality sequences were trimmed and filtered. The operational taxonomic units (OTUs) clustered at 97% similarity for the remaining high-quality sequences, and their taxonomy was assigned on the basis of the Human Oral Microbiome Database. The Shannon, Chao1, observed species, and phylogenetic diversity (PD) whole-tree indexes of alpha diversity were calculated to estimate the microbial community diversity. Beta diversity and principal coordinate analysis (PCoA) plots were generated to assess the microbial community composition. The relative abundances at each taxonomic level were calculated. To compare the microbial communities among the time points, we generated the microbial distribution at the genus and phylum levels. Statistical analyses of clinical parameters were done using SPSS software (version 20.0; IBM Corp, Armonk, NY). The significance of differences among time points were evaluated using repeated-measures analysis of variance.

RESULTS

The overall mean PPD was 2.49 mm at T0, 2.77 mm at T2, and 2.75 mm at T3. The overall mean GI was 1.68 at T0, 1.98 at T2, and 1.75 at T3. The PPD and GI increased slightly, but not significantly, at T2. After appliance placement, the PLI significantly increased between T0 and T1 (1.1 and 2.1, respectively), followed by a decrease from T2 to T3 (1.3 and 1.0, respectively) (Fig 1). In subgroup analyses, the PPD and GI of both high-risk sites and low-risk sites showed a slight increase at T2 without statistically significant difference (Fig 2). The mean PPD at the high-risk site was 2.78 mm at T0, 2.99 mm at T2, and 2.98 mm at T3, whereas the corresponding mean PPD at the low-risk site was 2.30 mm, 2.62 mm, and 2.60 mm, respectively. The mean GI at the high-risk site was 1.72 at T0, 1.93 at T2, and 1.75 at T3, compared with 1.65 at T0, 1.95 at T2, and 1.76 at T3 in the low-risk sites.

A total of 1,070,328 raw reads was obtained (mean, 35,677; SD, 13,225). The trimming and filtering of the raw reads led to the generation of 920,709 clean reads (mean, 30,690; SD, 10,803). The length of 99.98% of the clean reads was 400 to 440 base pairs. OTU analyses showed that 246 OTUs were obtained at T0, 258 OTUs at T1, and 258 OTUs at T2. Venn analyses showed that 228 OTUs were common at 3-time points.

The alpha diversity was analyzed by calculating the chao1, observed species, shannon and PD whole-tree indexes (Fig 3). Compared with T0, the chao1, observed species, and PD whole-tree indexes increased at T1, albeit not significantly.

Beta diversity was analyzed by calculating the weighted UniFrac distance and performing a PCoA. The distance between groups was measured on the basis of similarities in the microbial structure. The weighted UniFrac distance decreased at T1, but the weighted UniFrac distances at T0 and T2 were similar (Fig 4). The PCoA indicated some overlaps among the 3 groups, which could not be clearly separated from each other (Fig 5).

The composition of the dominant microbiome (relative abundance >1%) was analyzed at the phylum and genus levels (Fig 6). At the phylum level, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria* were found at all 3-time points and accounted for about 97.99% of the total sequences. Among these, the relative abundance of *Bacteroidetes* and *Actinobacteria*

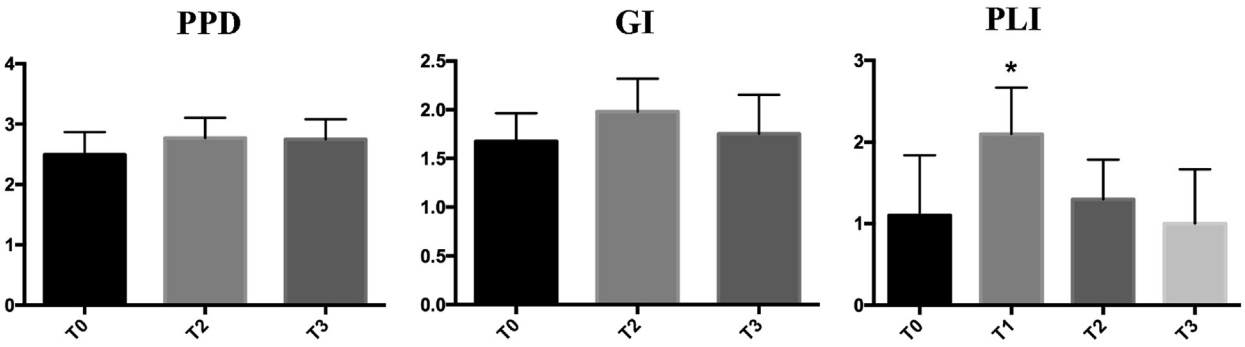

Fig 1. The overall periodontal status of patients with periodontitis during orthodontic treatment. The PLI was significantly greater at T1 than at the other 3 time points. The GI and PPD were not significantly different among the time points. Data are represented as mean ± SD. $P < 0.05$ was considered statistically significant using the repeated-measures analysis of variance.

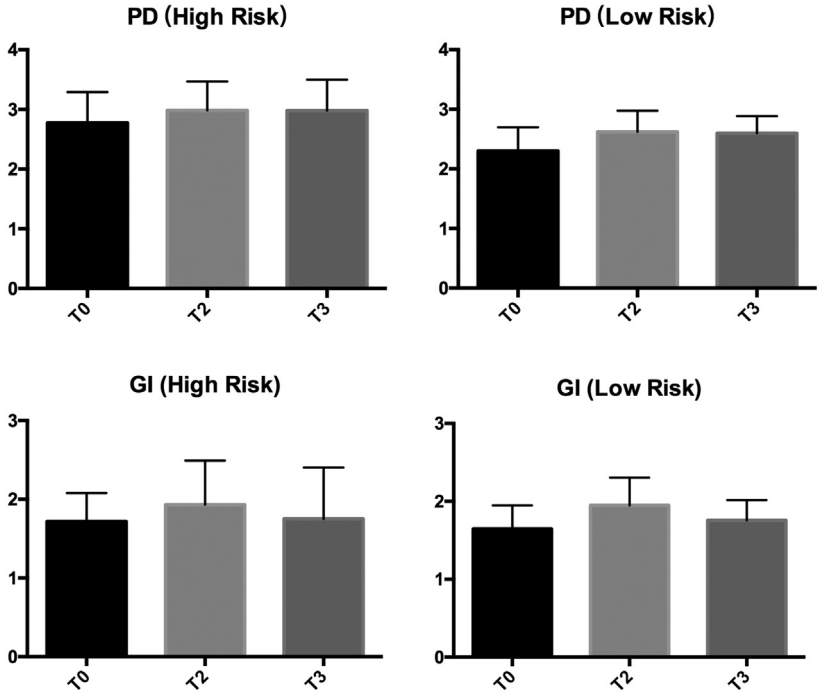

Fig 2. The subgroup analyses: the PPD and GI of high-risk sites and low-risk sites. Both PPD and GI in the 2 subgroups showed a similar trend, a slight increase at T2 without statistically significant difference. $P < 0.05$ was considered statistically significant using the repeated-measures analysis of variance.

showed a transient change at T1. The relative abundance of *Bacteroidetes* at T0, T1, and T2 was 19.77%, 10.60%, and 17.39%, respectively. The relative abundance of *Actinobacteria* at T0, T1, and T2 was 6.65%, 10.63%, and 7.01%, respectively. At the genus level, the relative abundance of *Rothia* (5.29% at T0, 8.50% at T1, and 5.35% at T2), *Prevotella* (10.43% at T0, 2.99% at T1, and 5.83% at T2), *Streptococcus* (18.01% at T0, 18.92% at T1, and 14.40% at T2), and *Haemophilus* (12.83% at T0, 17.25% at T1, and 13.21% at T2) changed slightly. The microbial composition at the phylum and genus levels was similar at T0 and T2.

The core microbiome was defined as the OTUs with a prevalence >100%. Five core genera and 26 core species were found in all of the groups at the 3-time points. At the genus level, *Neisseria* was the most abundant core genus, followed by *Leptotrichia*, *Gemella*, *Lachnoanaerobaculum*, and *Oribacterium* (Fig 7, *A*). The relative abundance of these core genera was stable during the first 3 months of orthodontic treatment. Six species, *Prevotella nanceiensis*, *Campylobacter rectus*, *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia,* and *P intermedia*, were associated with periodontal disease (Fig 7, *B*). The relative abundance of these 6 periodontal pathogens was stable during the first 3 months of orthodontic treatment but varied among individual patients.

DISCUSSION

An increasing number of patients with periodontitis seek orthodontic treatment to improve their aesthetics and masticatory function. Orthodontic treatment is indispensable, particularly for pathologic tooth migration. Inflammation should be controlled during the movement of periodontally compromised teeth, which should be induced by an appropriate level of orthodontic force.[6] The periodontal changes that occur in patients with periodontitis during orthodontic treatment are a concern for both orthodontists and periodontists. Most prior studies have investigated the microbial or clinical changes that occur in patients without periodontal disease during orthodontic treatment[15,16]; however, few have focused on effects on the oral microbiome of periodontal orthodontic treatment. Hence, we used next-generation pyrosequencing to evaluate the microbial community of patients with periodontitis and its relationship with clinical change.

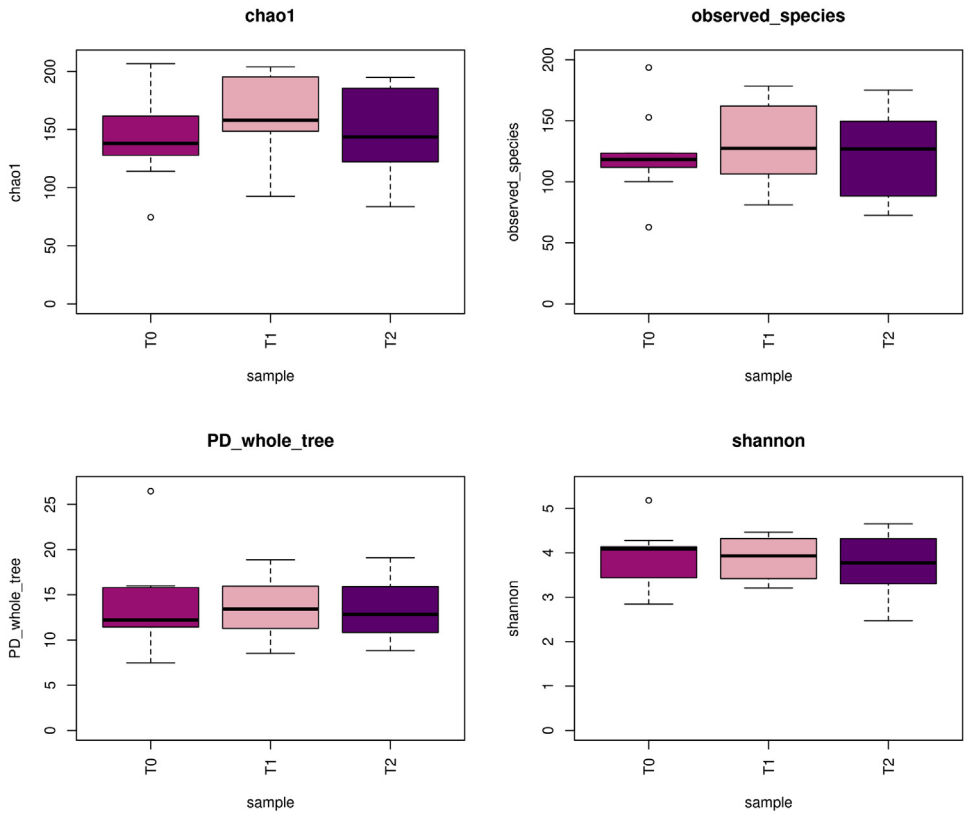

Fig 3. Alpha diversity indexes of the saliva microbial community at T0, T1, and T2. The chao1, observed species, and PD whole-tree values increased at T1, albeit not significantly.

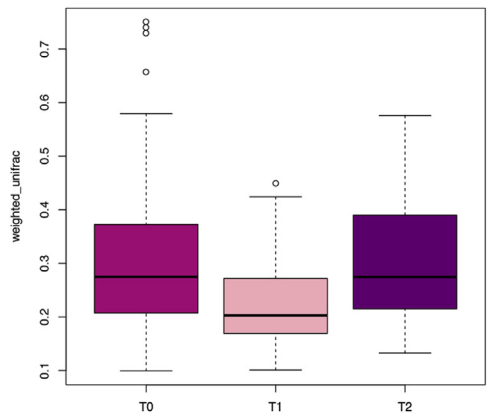

Fig 4. Weighted UniFrac distances (beta diversity) at T0, T1, and T2. The beta diversity decreased at T1, but not significantly.

Plaque accumulation is related to the development of periodontal disease.[7] During the first month of orthodontic treatment, the PLI was significantly increased. Thus, the microbial community composition at 1 month after appliance placement was different from that before appliance placement. The microbial community diversity was slightly increased at T1, which was associated with an unhealthy status. The PLI returned to baseline at 3 months. Hence, the microbial community composition at T2 was similar to that at T0, and the diversity returned to the baseline level. The stability of the microbial community plays an important role in periodontal health.[17] Microbial community diversity and composition, which could reflect the transition of periodontal status, was mainly focused in our study. Therefore, the host-microorganism balance was altered at 1 month after appliance placement, as indicated by the altered microbial community diversity and structure. The host-microorganism balance was reestablished at 3 months after appliance placement. Patients with severe periodontitis receiving maintenance periodontal treatment typically have good compliance with oral health procedures. At 1 month, the patients were unfamiliar with oral hygiene maintenance using brackets, so the PLI transiently increased. Once they became familiar with oral hygiene maintenance using brackets, plaque control was achieved, and the host-microorganism balance was reestablished. Hence, plaque control is necessary

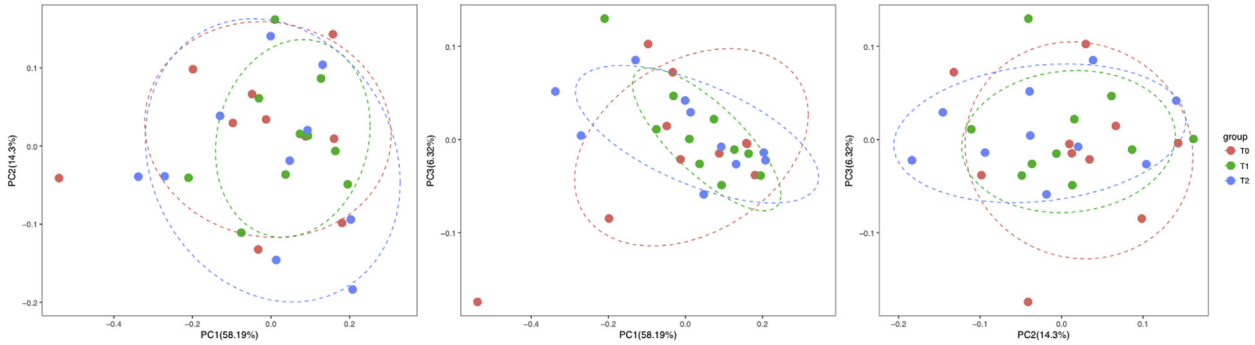

Fig 5. PCoA of the community structure of salivary samples at T0, T1, and T2. The communities at T0, T1, and T2 could not be clearly separated from each other.

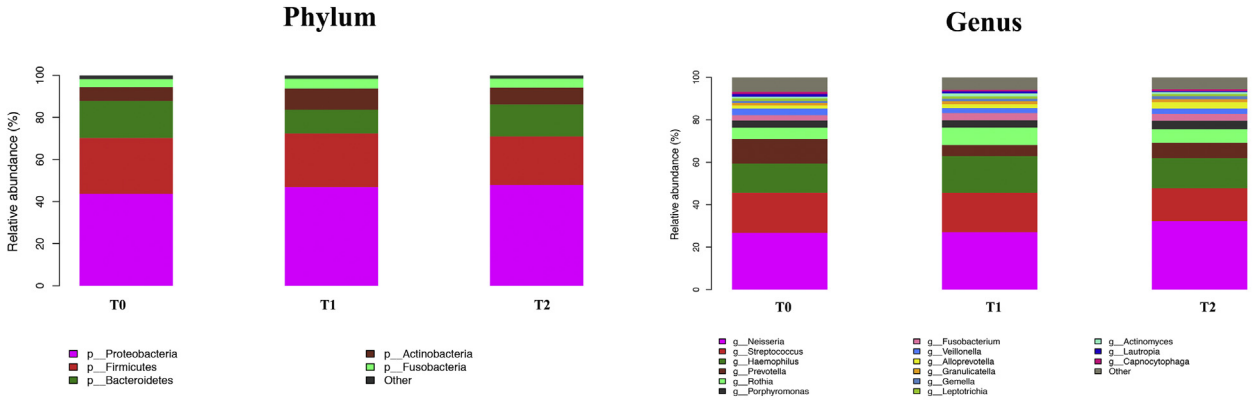

Fig 6. Distribution of the dominant microbiome (relative abundance > 1%) at the phylum and genus levels. At both the phylum and genus levels, the microbial distribution at T0 and T2 was similar.

for patients with periodontitis during orthodontic treatment. The PLI should be evaluated, and the use of plaque indicators is recommended, particularly in the first stage of orthodontic treatment.

Metal corrosion caused by the placement of orthodontic appliances can modulate the host-microorganism balance. The nickel and chromium concentrations in saliva reportedly significantly increase during orthodontic treatment.[18] Speer et al[19] reported that the metal ions released from the appliance and archwire, particularly nickel ions, may have toxic effects on microorganisms. Besides the metal appliance, metal-based restoration may have also a toxic effect on the composition of the microbial community.[20] By contrast, a variety of metal ions are required for the metabolism of several microbial taxa. The periodontal pathogen, *P intermedia*, needs iron for survival and reproduction.[21] In this study, the relative abundance of *P intermedia* slightly, but not significantly, increased. The effects of metal corrosion on the salivary microbiome warrant further investigation.

We also analyzed the core microbiome (an indicator of microbial community structure[22]) and a variety of periodontal pathogens. The core microbiome, which is most common and shared, could influence the microbial community. In our study, 6 core genera were detected and remained stable. The core microbiome was less affected by the orthodontic appliance. Hence, the microbial community might be stable. Several previous studies have assessed the relative abundance of periodontal pathogens during orthodontic treatment.[16,23] We detected 6 species reported to be periodontal pathogens. Despite the marked intersample variation, the abundance of the periodontal pathogens remained stable during orthodontic treatment.

The overall PPD and GI did not significantly change, and the periodontal status was healthy during the first 6 months of orthodontic treatment. In addition, the

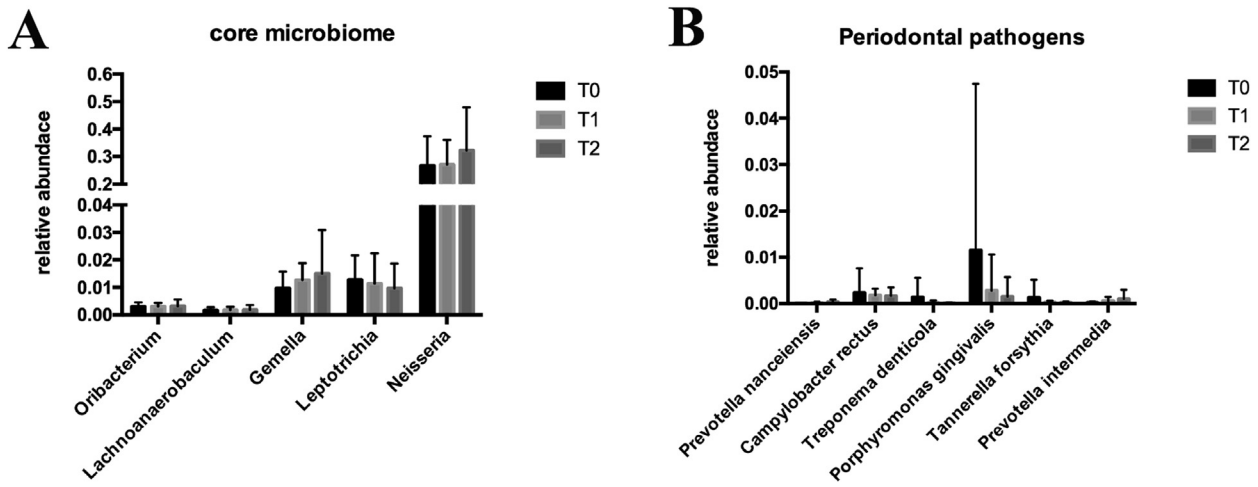

Fig 7. The relative abundance of the 5 core microbiomes at the genus level and 6 periodontal pathogens at the species level. **A,** As an indicator of microbial community structure, the relative abundance of 5 core genera was relatively stable. **B,** The relative abundance of these 6 periodontal pathogens associated with periodontal disease did not significantly increase. Data are represented as mean ± SD. $P < 0.05$ was considered statistically significant using the repeated-measures analysis of variance.

PPD and GI of both the high-risk site and low-risk site were relatively stable. Once the periodontal inflammation was under control, orthodontic treatment did not induce inflammation relapse regardless of the level of original attachment loss. The PPD and GI showed a transient increasing trend, albeit later than in the PLI and microbial community. This finding could be due to the lag effects of periodontal tissue on the plaque accumulation and microbial community. The significantly increased plaque accumulation and slightly increased GI in the early stage of orthodontic treatment suggest transient mild gingivitis. This result was supported by the return of the microbial community of saliva and clinical status to baseline levels, in accordance with our previous systematic review.[11]

The microbial and clinical changes that occur in orthodontic patients with severe periodontitis are similar to those in periodontally healthy orthodontic patients.[24] Severe periodontitis is not a contraindication for orthodontic treatment. Among adult patients, including those with healthy but reduced periodontal tissue, appropriate orthodontic treatment with strict plaque control does not cause irreversible damage to periodontal tissue. Several clinical studies have shown that periodontal lesions can be repaired by orthodontic treatment, and the long-term results are stable. The underlying mechanisms are thought to be a reduction of inflammation and application of an appropriate level of orthodontic force. In periodontally compromised patients, the center of resistance of the teeth moves apically because of loss of alveolar bone. Hence, inappropriate orthodontic treatment has detrimental effects on the periodontal tissue. Inflammation was controlled in all of the patients in this study before they began orthodontic treatment and was treated by a periodontal orthodontic specialist.

To the best of our knowledge, this is the first study of the effects of orthodontic treatment on the salivary microbial community of patients with periodontitis. Speer et al[19] reported that in patients with periodontitis, the total bacterial count increased, and the growth of periodontal pathogens was inhibited during orthodontic treatment. Interestingly, the GI was found to decrease 6 weeks after appliance placement. They attributed the microbial and clinical change to metal corrosion. By contrast, GI increased 1 month after appliance placement in our study. Culture-based detection of periodontal pathogens was employed in the prior study, so the microbial structure could not be analyzed. In addition, the low relative abundance of microbial pathogens does not necessarily indicate periodontal health. The change in microbial structure is more important than that in several traditional pathogens.[25] Hence, we used next-generation pyrosequencing to assess the microbial community.

Risk assessment of periodontitis patients before and during orthodontic treatment is essential. Regular periodontal examinations, including probing and radiographic imaging, are commonly used during orthodontic therapy. However, PPD and GI might not be useful for the early diagnosis of periodontal disease. Early detection of periodontal inflammation is essential, in which the microbiome, an etiologic agent of

periodontal disease, should play an important role. The salivary microbiota has been used as an indicator to monitor the oral microflora and periodontal status.[26,27] Kim et al[28] developed a grading system for the relative abundance of 9 periodontal pathogens in saliva to enable early detection of periodontitis. At present, the cost of microbiologic analysis cannot be ignored, and the microbial examination is not widely used in the clinic. In the future, a new method or analysis kit to detect limited kinds of microbiota might be economical. Considering the high-expense microbial sequencing and lack of severe periodontitis patients asking for orthodontic treatment, the small sample size hampered our ability to detect changes in the microbiome; thus, future studies should involve a larger sample.

CONCLUSIONS

Within the limitations of this preliminary study, the orthodontic appliance promoted plaque accumulation and changed the microbial community during the first month of orthodontic treatment. However, the host-microorganism balance was restored at 3 months after appliance placement, and the periodontal inflammation during the 6-months orthodontic treatment was under control. Further studies with a larger sample are required to verify our findings.

AUTHOR CREDIT STATEMENT

R.G. performed the experiments, analyzed the data, drafted the paper, prepared figures, reviewed drafts of the paper. J.S. performed the orthodontic treatment for including patients and reviewed drafts of the paper. L.Z. contributed to the analysis of sequencing data. Y.Z. and W.L. conceived and designed the experiments, reviewed drafts of the paper.

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