Title

The Oral Microbiome and Clinical Changes in Patients with Fixed and Clear Aligner Orthodontic Appliance after Debonding

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The Oral Microbiome and Clinical Changes in Patients with Fixed and Clear Aligner Orthodontic Appliance After Debonding

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science in Oral Biology

by

Oman Konia

2023

ABSTRACT OF THE THESIS

The Oral Microbiome and Clinical Change in Patients with Fixed and Clear Aligner Orthodontic Appliance After Debonding

by

Oman Konia

Master of Science in Oral Biology

University of California, Los Angeles, 2023

Professor Renate Lux, Chair

Background:

The most popular method for addressing malocclusion is by using fixed or removable orthodontic devices. However, these appliances can inadvertently accumulate plaque shortly after their application, leading to an imbalance in the bacterial population. This bacterial

dysbiosis could increase the risk of several health issues, including dental caries, white spot lesions, periodontal diseases, and even metabolic diseases. This research investigates the changes in the gingival and plaque indices and the bacterial composition following the removal of orthodontic devices. Moreover, this work explores the ambiguity surrounding whether microbiological and clinical changes experienced by patients during orthodontic treatment are reversible post-therapy and compares the changes for fixed and clear aligner Orthodontic appliances.

Objective: To analyze the initial changes in clinical parameters and plaque levels of periodontal pathogens after orthodontic treatment with fixed or removable appliances.

Materials and Methods: The subjects comprised 14 patients completing orthodontic treatment ready for debonding. The Plaque Index and Gingival Index were measured as periodontal parameters. The plaque and gingival indexes were obtained from both arches' central incisors, lateral incisors, canines, and first molars. Plaque and periodontal parameters were obtained at the following three-time points: at debonding (T0), one month (T1), and three months (T2) after debonding. Deep 16S rRNA sequencing was performed for the supra gingival plaques to analyze the correlation with the clinical indexes and to see the microbial community composition after debonding at 3-time points (T0, T1, and T2).

Results: The Fixed appliance (FA) group had a statistically significant greater overall GI score than the Clear aligner (CA) group at time T0 ($p<0.01$) and T2 ($p<0.01$). The FA group also had a statistically significant greater anterior and posterior GI score at T0 ($p<0.01$) and T2 ($p<0.05$) as compared to the CA group. Similarly, the patients with Fixed appliances generally showed

statistically significantly greater overall, anterior, and posterior PI scores at all time points T0 ($p<0.01$), T1 ($p<0.05$), and T2 ($p<0.05$) as compared to the CA group. A greater reduction in plaque index was observed for the FA group from T0-T2, which was statistically significant at $p<0.01$ as opposed to the CA group.

The microbiological analysis revealed no correlation between the relative abundance of different microbes and the timepoint after debonding for either the FA or CA group. However, the FA group had higher PI and GI scores relative to the CA group. Further, the FA group showed higher levels of *Neisseria* and *Veilonella,* which are known to be health-associated bacteria. Conversely, the CA group was observed to be associated with *Neisseria, Capnocytophaga, and Leptotrichia* at the lower plaque and gingival index scores with respect to the FA group.

Alpha – and beta-diversity showed no significant differences in the composition of supragingival plaque when correlated with the Plaque index, Gingival Index, and the various time points.

Conclusion: This study's findings align with prior research, indicating changes in clinical indices after orthodontic devices are placed in the mouth. We observed a general trend towards reduced clinical index parameters after the removal of both types of orthodontic appliances. Patients with fixed appliances showed higher Gingival and Plaque indices than those with clear aligners, although these indices decreased over time for both groups. Our microbial evaluation revealed higher levels of *Neisseria* and *Veilonella*, both generally associated with oral health, in the fixed appliance group. Meanwhile, the clear aligner group demonstrated links with *Leptotrichia, Capnocytophaga*, and *Neisseria* at the lower plaque and gingival index scores. Notably, our

analysis did not find any correlation with harmful bacteria related to periodontal disease or dental caries after removing Orthodontic appliances in either group.

The thesis of Oman Konia is approved.

Sanjay M. Mallya

Jimmy Kuanghsian Hu

Renate Lux, Committee Chair

University of California, Los Angeles

2023

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INTRODUCTION

The oral cavity is the second most diverse ecosystem in the body and harbors up to 772 prokaryotic species of microorganism.[1] Maintaining homeostasis within the oral microbiota is essential for overall oral and general health. Dysbiosis of this complex microbial ecosystem may contribute to the onset of various oral pathologies as well as systemic diseases.[2] Many studies have found correlation between the oral microbiome and pulmonary diseases,[3] cardiovascular diseases,[4] rheumatoid arthritis,[5] Alzheimer's disease,[6] and other metabolic diseases.[7]

It's widely recognized that orthodontic treatment is the most used approach to correct malocclusion and dentofacial anomalies. Nonetheless, starting orthodontic treatment, whether through traditional braces or clear aligner therapy, can make brushing and flossing challenging, leading to a significant accumulation of plaque and biofilm.[8] The accumulation of plaque due to orthodontic treatment, whether with fixed or removable appliances, can lead to adverse effects like gingival inflammation and enlargement.[9-11] Orthodontic brackets in particular, can serve as a harbor for oral bacteria, causing an increase in plaque buildup and gingival issues, thereby altering the oral microbiota following the start of orthodontic therapy.[12] It has been known that *Streptococcus mutans* is the most common microorganism involved in the initiation of caries, whereas lactobacilli are frequently involved in dental caries progression.[13] Previous studies have noted a significant increase in *Streptococcus mutans* and *Lactobacillus* species in dental Plaque and saliva after initiation of Orthodontic treatment.[14, 15] Many studies have also evaluated most periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia* [16]*, Porphyromonas gingivalis, and Tannerella forsythia,* which are strongly related to gingival inflammation and periodontal destruction, and are significantly

increased in patients after initiation of orthodontic treatment.[17] Understanding changes in the levels of harmful bacteria during and post orthodontic treatment is crucial, as these alterations are associated with overall oral health, including issues such as periodontal diseases and tooth demineralization.

Previous studies have shown a positive association between the levels of periodontal pathogens in saliva and subgingival plaque.[18] Considering dental plaque collection is a simple, safe, economical, and non-invasive method, it could be a suitable medium for monitoring oral pathogen levels after orthodontic treatment. Many studies have reported quantitative changes in bacterial levels with initiation of orthodontic treatment with fixed or removable orthodontic appliances.[14, 15, 19-23] Limited research exists that examines changes in the oral microbiome following the removal of orthodontic appliances, with very few studies focusing on clinical or microbial transformations post-debonding. The available findings are inconclusive and ambiguous, leaving uncertainty about the post-debonding microbial shifts. Moreover, whether these organisms decrease or increase in a patient solely due to them undergoing orthodontic treatment is unclear as well.[24-26]

Hence, this prospective, in vivo study aims to assess the clinical changes in supra-gingival plaque levels, using gingival and plaque indices, and to conduct a microbial examination of different bacterial genera. This analysis compares the outcomes between two groups: those undergoing Fixed Appliance (FA) therapy and those using Clear Aligner (CA) therapy, post-orthodontic treatment (which is termed as debonding in Orthodontics).

We predict a decrease in the plaque and gingival scores with increasing time after debonding. Clinically, a decrease in gingival inflammation, gingival enlargement, and bleeding upon probing is noticeable after the removal of braces. We hypothesize that a beneficial shift in the microbial environment will likely be observed during the retention phase following brace removal as these clinical symptoms improve. [27] Furthermore, we additionally hypothesize that the reduction in clinical parameters will be greater in patients with fixed appliances as compared to those with clear aligners. This is due to the tendency of fixed appliances to accumulate more plaque than clear aligners. [28] In the future, we can also correlate the microbial change with specific parameters like the type of retainer (removable Hawley, wraparound retainer, and fixed lingual bonded retainer) and duration of orthodontic treatment.

Patients who pursue orthodontic treatment dedicate substantial time and resources to improve the appearance and alignment of their teeth. Enhancing our understanding of the oral microbiome following the removal of braces will provide insights into the nature of microbial changes. Additionally, it can guide us in creating an oral hygiene regimen that specifically addresses any elevated microorganisms, ensuring we provide the highest level of care to our patients.

Overall objective and specific aims:

Objective: This study aims to evaluate the change in clinical indices and microbial shift in supra gingival plaque in patients with fixed and clear aligner orthodontic appliances after debonding.

Specific aims:

1. To evaluate and compare the Plaque and gingival indices at 3-time points- at debond (Baseline/T0), 1 month (T1) and 3 months (T2) after debond.
2. To characterize and compare plaque microbiota via deep-sequencing using the Illumina MiSeq platform and the Human Oral Microbiome Database at 3-time points- at debond (Baseline/T0), 1 month (T1), and 3 months (T2) after debonding of the Orthodontic appliance.

Materials and method

The subjects consist of patients who finished orthodontic treatment at UCLA Orthodontics clinic and are ready to be debonded. Inclusion and exclusion criteria were applied to all debond cases to determine the samples for this study. The recruitment goal according to the power analysis was known to be 40.

Inclusion criteria-

1. Subjects completing orthodontic treatment at UCLA Orthodontics clinic.
2. Male and females, 12 years and older
3. Assent and informed consent signed.
4. Subjects must have undergone complete orthodontic treatment- with either fixed or removable orthodontic appliance.
5. Subjects should come for regular retainer checks after completion of treatment.

Exclusion criteria-

1. Active caries
2. Advanced periodontal disease
3. Patients with chronic systemic diseases
4. Patients who currently used or have used antibiotics in the last 30 days.
5. Patients with significantly reduced saliva production and patients who have had radiation therapy to the head and neck region.

On the day of the debond, patients were briefed about the study, and patient consent was obtained for agreement of participation. Plaque and gingival indices were noted along with supragingival plaque samples collection at 3 time points- at debond (T0), 1 month (T1) and 3 months (T2) after debond.

For plaque index, supragingival Plaque was scored using the Turesky et al. Modified Quigley-Hein Plaque Index (TQHPI) as seen in (Table 1)[29-31].

Table 1: Criteria for the Turesky et al. Modified Quigley- Hein Plaque Index (TQHPI) [31]

PI Scores	Criteria
0	No plaque
1	Separate flecks of plaque at the cervical margin of the tooth
2	A thin continues band of plaque (up to one mm) at the cervical margin of the tooth
3	A band of plaque wider than one mm but covering less than one-third of the crown
4	Plaque covering at least one-third but less than two-thirds of the crown of the tooth
5	Plaque covering two-thirds or more of the crown of the tooth

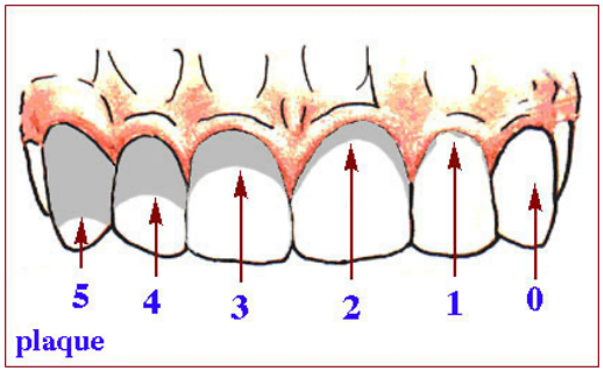


Figure 1: Turesky et al. Modified Quigley- Hein Plaque Index [31]

For gingival index, clinical signs of gingival inflammation like bleeding and swelling of gingiva were assessed using the Löe and Silness Gingival Index (GI) (Table 2)[32, 33]. The GI does not consider the periodontal tissues' quantitative changes, such as pocket depths, but instead focuses on the qualitative changes [34].

Table 2: Loe and Silness Gingival Index Scoring System[34]

GI Scores	Gingival Status	Criteria
0	Normal Gingiva	Natural coral pink gingiva with no evidence of inflammation
1	Mild Inflammation	Slight changes in color, slight edema. No bleeding on probing.
2	Moderate Inflammation	Redness, edema and glazing. Bleeding upon probing
3	Severe Inflammation	Marked redness and edema/ulceration/tendency to bleed spontaneously

For plaque collection, Supragingival Plaque were scraped using a dental scaler from buccal surfaces of all incisors, premolars and firs molar and deposited into sterile plastic collection tubes. All patients in the clinic got a temporary, clear retainer called Essix on the day of debonding and Hawley retainer 1 month after.

All plaque samples were deposited into separate sterile collection tubes containing in 500 uL phosphate-buffered saline (PBS). DNA from the plaque samples was extracted. Microbial community analysis was performed via next-generation sequencing of the V4 region of the 16S rRNA gene using the MiSeq platform (Illumina) available at the UCLA Microbiome Core [35]. Bioinformatic data analysis was performed using the following procedures: after demultiplexing

and trimming of barcodes, low-quality sequences containing bases with Phred quality values <20 and sequences with >3% uncertain base pairs were removed. The 16S rRNA sequences were clustered into operational taxonomic units at a 98% similarity level using QIIME2 [36]and taxonomically assigned by comparison to the Human Oral Microbiome Database (HOMD) [37]. Alpha-diversity (Shannon Index), Beta-diversity (Weighted UniFrac), and principal coordinate analyses were calculated in QIIME2. The power of this study was calculated using the G*Power statistical analysis program [38, 39]. The normality of the data was determined using the Shapiro-Wilk analysis. Statistical significance was calculated using t-test for the PI data and the Mann Whitney U-test for the GI data at a level of $p \leq 0.05$.

Results

The mean age of the patients in the fixed appliances group was 18.27 years (SD= 4.09 years). The mean age of the clear aligner group was approximately 5 years older at 23.82 years (SD= 5.74 years). The difference in the age of the participants in each group is of marginal statistical significance (p= 0.076) and could be considered a limitation in this study. The fixed appliance group was composed of eight patients, five of them were male and three were female. The clear aligner group contained a total of six patients, two of them were male and four of them were female. The demographics for the patient in the study are given in table 3 and are depicted graphically in figure 2.

Table 3: Patient number, gender distribution and mean ages compared between the fixed appliance and clear aligner groups.

	Fixed Appliances	Clear Aligners
Subjects	8	6
Male	5	2
Female	3	4
Mean Age	18.28	23.82
SD	4.10	5.74

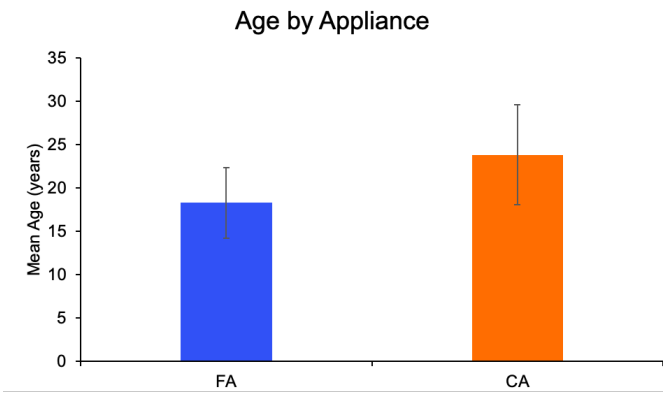


Figure 2: Mean ages compared between the fixed appliance and clear aligner groups. The fixed appliances group is shown in blue, and the clear aligner group is shown in orange. The mean age for the clear aligner group is approximately 5 years older than the mean age for the fixed appliance group.

As illustrated in Figure 3, the FA group had a statistically significant greater overall GI score than the CA group at time T0 ($p<0.01$) and T2 ($p<0.01$). The FA group also had a higher overall GI score than the CA group at time T1, however the p-value was not significant ($p=0.08$), possibly due to the limited sample size of the study. Despite the significant differences, the GI decreased over time albeit not significantly for both groups.

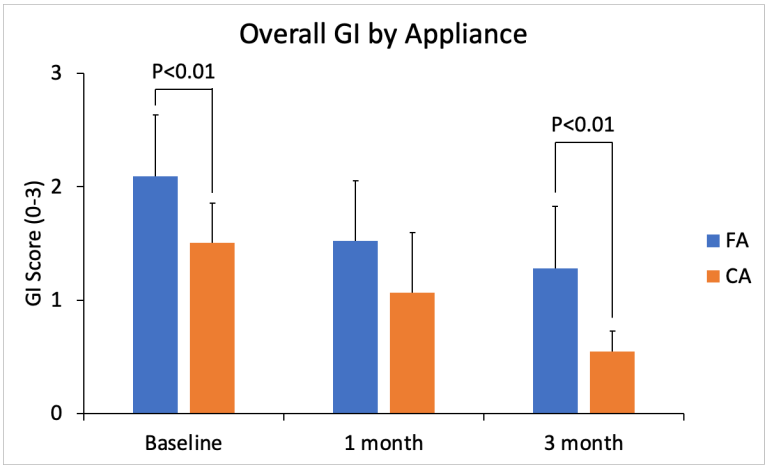


Figure 3: Overall gingival index score comparing fixed appliances to clear aligners over each time point.

Figure 4 shows the decrease in overall GI scores at time points T1 and T2 relative to the overall GI score at time T0 for both groups. The equations for these calculations are shown below in equations (1) and (2)

$$1\ \text{month} - \text{baseline} = \text{Overall\_GI\_score}(T0) - \text{Overall\_GI\_score}(T1) \quad (1)$$

$$3\ \text{month} - \text{baseline} = \text{Overall\_GI\_score}(T0) - \text{Overall\_GI\_score}(T2) \quad (2)$$

In general, the rate of decrease in overall GI scores with respect to time was similar between both groups, but slightly higher in the FA group. At time T1 the mean reduction in overall GI scores was 0.57 in the FA group and 0.44 in the CA group. This was not a statistically significant difference ($p=0.44$). Likewise, the mean reduction at time T2 was 0.96 and 0.82 for the FA and CA groups respectively, which was also not a statistically significant difference ($p=0.34$).

As the FA group had a larger baseline overall GI score at time T0 and the rate of decrease in overall GI scores was not statistically significant between the groups, it follows that the FA group had a higher overall GI score throughout the course of this study.

Moreover, the data was stratified to test for any significant differences between the GI score in the anterior portion of the mouth (from the incisors and canines) as well as the posterior portion of the mouth (premolars and molars).

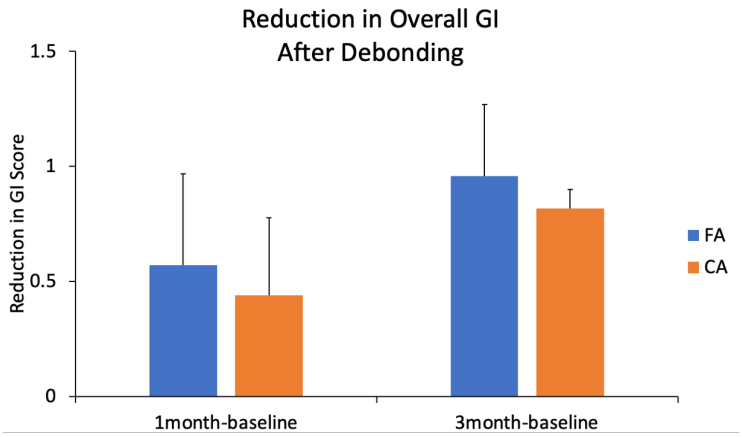


Figure 4: Reduction in Overall gingival index after debonding

Figure 5 shows how the anterior GI score varied in both groups at each time point. Similar to the above findings for the overall GI score, the FA group had a larger Anterior GI score than the CA group at all time points. The difference in Anterior GI was a statistically significant difference with a p-value of 0.01 and 0.03 at times T0 and T2, respectively.

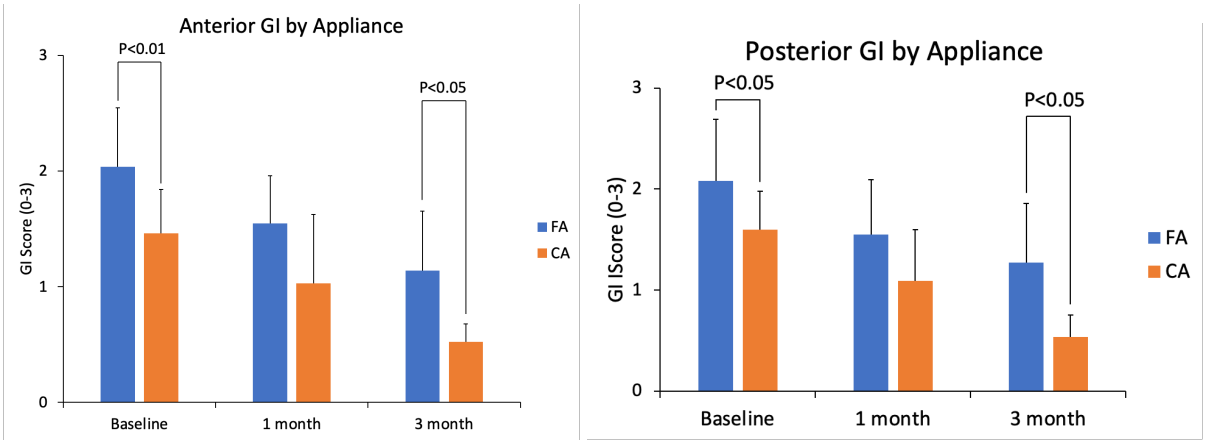


Figure 5: Anterior and posterior gingival indices comparing fixed appliances to clear aligners over each time point.

Figure 5 also shows how the posterior GI score varied in both groups at each time point. Consistent with the findings of both the overall GI and Anterior GI scores, the FA group had a larger Posterior GI score than the CA group at all time points. The difference in Posterior GI scores between the FA and CA groups had a statistically significant p-value <0.05 for times T0 and T2. For time point T1 the p-value was of marginal significance with a value equal to 0.07.

Figure 6 shows the decrease in Anterior GI scores between time points T1 and T2 relative to the Anterior GI score at T0 for both groups. The equations used in this calculation are shown in (3) and (4) below.

$$1\ \text{month} - \text{baseline} = \text{Anterior\_GI\_score}(T0) - \text{Anterior\_GI\_score}(T1) \quad (3)$$

$$3\ \text{month} - \text{baseline} = \text{Anterior\_GI\_score}(T0) - \text{Anterior\_GI\_score}(T2) \quad (4)$$

Like the overall GI score, the decrease in Anterior GI scores with respect to time was similar in both groups, but slightly higher in the FA group. At time T1 the mean reduction in Anterior GI scores was 0.49 in the FA group and 0.43 in the CA group. This difference was not statistically significant (p=0.73). Similarly, the mean reduction at time T2 was 0.94 for the FA group and 0.80 for the CA group which was not statistically significant (p=0.36).

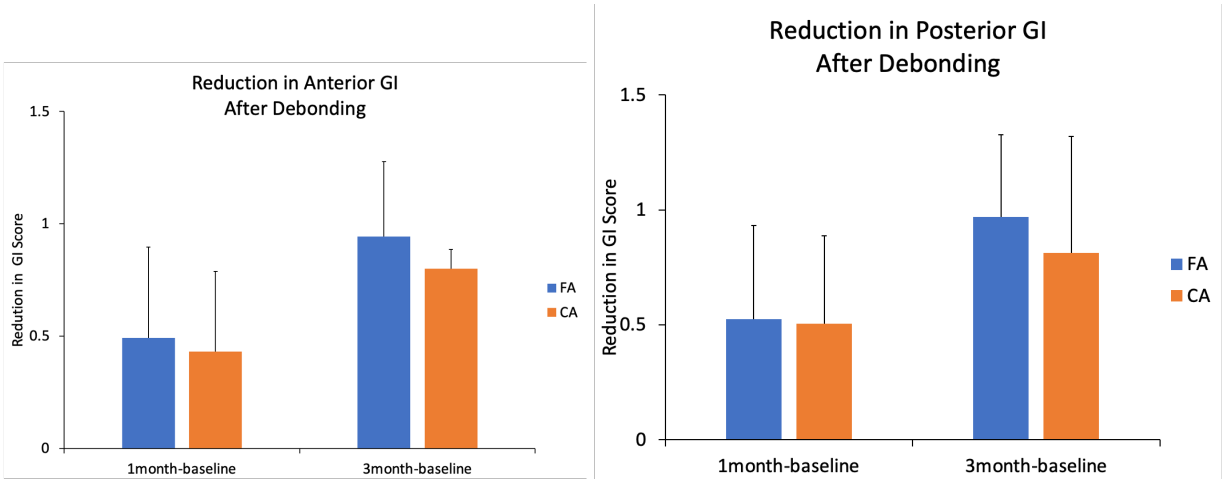


Figure 6: Reduction in Anterior GI and Posterior GI after debonding of appliance

Figure 6 also shows the decrease in Posterior GI scores between time points T1 and T2 relative to the Posterior GI score at T0 for both groups. The equations used in this calculation are shown in (5) and (6) below.

$$1\text{ month} - \text{baseline} = \text{Posterior\_GI\_score}(T0) - \text{Posterior\_GI\_score}(T1) \quad (5)$$

$$3\text{ month} - \text{baseline} = \text{Posterior\_GI\_score}(T0) - \text{Posterior\_GI\_score}(T2) \quad (6)$$

Like the overall and Anterior GI scores, the reduction in Anterior GI scores with respect to time was similar in both groups, but slightly higher in the FA group at 3 months. At time T1 the mean reduction in Posterior GI scores was 0.50 in the FA group and 0.53 in the CA group. This was not a statistically significant difference ($p=0.89$). Similarly, the mean reduction at time T2 was 0.97 for the FA group and 0.81 for the CA group which was also not statistically significant ($p=0.33$).

Figure 7 depicts the Overall Plaque Index in FA and CA groups at 3 time points (baseline, T1 and T2). As demonstrated in Figure 7 the FA group had a statistically significant greater overall PI score than the CA group at baseline (p=0.01), at T1 (p= 0.02) and at T3 (p=0.05).

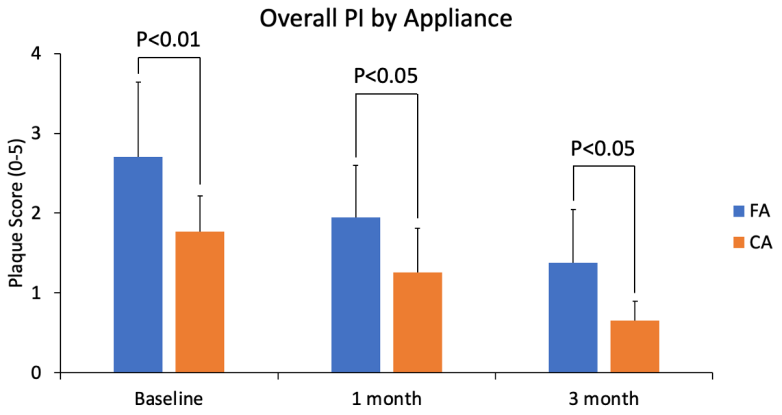


Figure 7: Overall plaque index score comparing fixed appliances to clear aligners over each time point.

Figure 8 shows the decrease in Overall PI scores at time points T1 and T2 relative to the overall PI score at baseline (time T0). We applied the same approach to calculate reductions that we previously utilized for GI in equations 1 through 6. In general, the decrease of PI score with respect to time was greater in the FA group relative to the CA group. At time T1 the mean reduction in overall PI scores was 0.76 in the FA group and 0.51 in the CA group. This was not statistically significant (p=0.14). At time T2 the mean reduction was 1.77 in the FA group and 0.84 in the CA group. This was a statistically significant difference with a p=0.01.

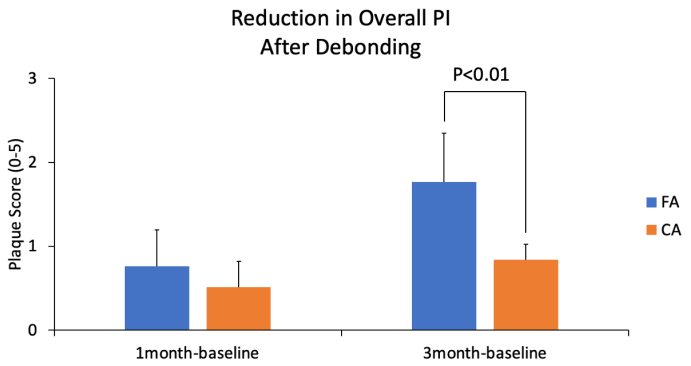


Figure 8: Reduction in overall PI after debonding of appliance

Figure 9 depicts the Anterior and Posterior Plaque index between FA and CA group at each point. Consistent with the Overall PI score, the FA group has a higher PI score as compared to CA group in both Anterior and posterior PI at all time points. There was a statistically significant difference in Anterior PI scores between FA and CA group at baseline (p=0.01), at T1 (p=0.03) and at T2 (p=0.04). The Posterior PI score also showed statistically significant difference at baseline (p=0.02), at T2 (p=0.01) and at T3 (p=0.05) between the FA and CA groups.

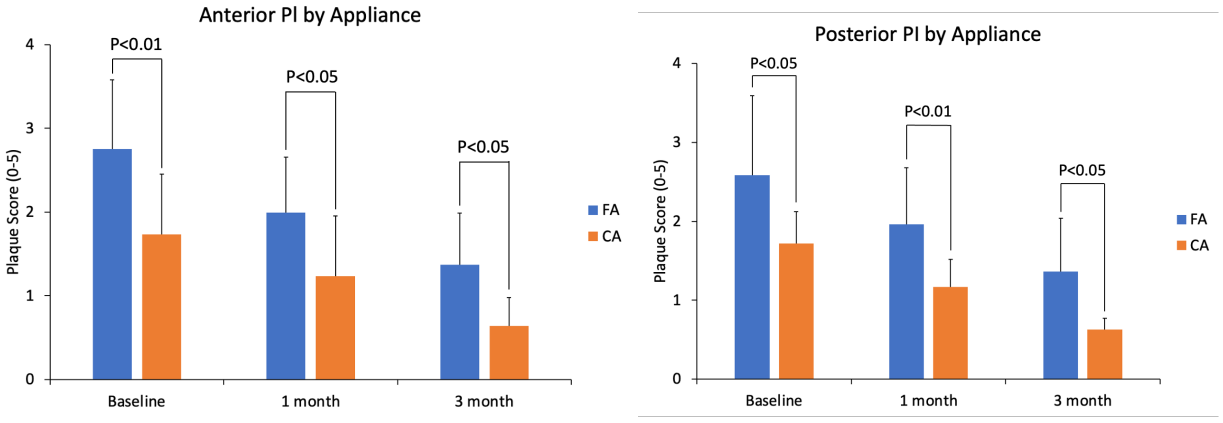


Figure 9: Anterior and posterior plaque indices comparing fixed appliances to clear aligners over each time point.

Figure 10 depicts the reduction in plaque scores between the two groups between time points T1 and T2 relative to baseline for the anterior and posterior plaque indices. The decrease in Anterior PI scores with respect to time was higher in the FA group. At time T1 the mean reduction in Anterior PI scores was 0.76 in the FA group and 0.50 in the CA group. This was not a statistically significant difference (p=0.11). The mean reduction at time T2 was 1.61 for the FA group and 0.85 for the CA group. This was a statistically significant difference with p-value of 0.05.

Like the Anterior PI scores, the reduction in posterior PI scores with respect to time was higher for the FA group as compared to the CA group. At time T1 the mean reduction in Posterior PI scores was 0.62 in the FA group and 0.55 in the CA group. This was not a statistically significant difference (p=0.65). However, the mean reduction at time T2 was 1.71 for the FA group and 0.82 for the CA group which was statistically significant (p=0.02).

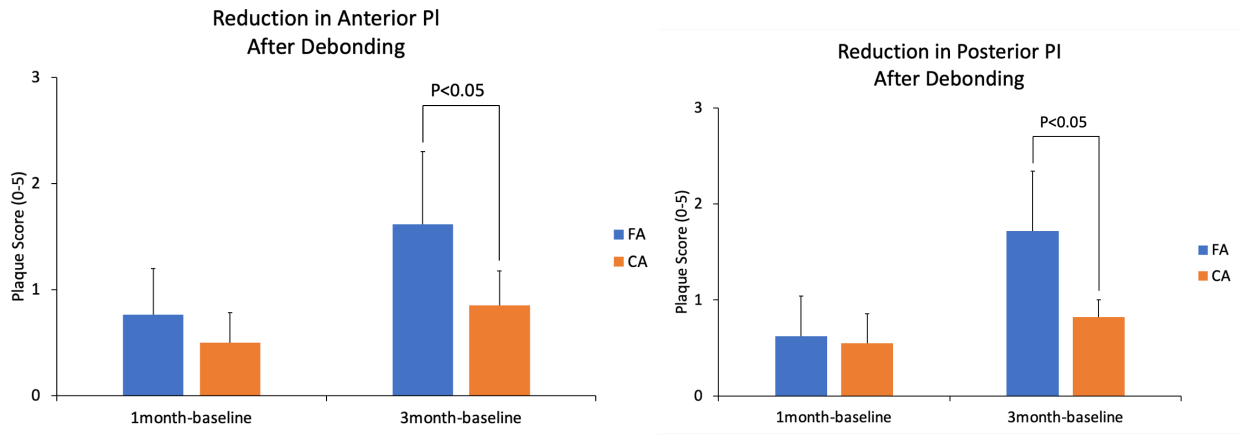


Figure 10: Reduction in Anterior Plaque Index and Posterior Plaque Index after debonding between baseline to 1 month and baseline to 3 months in FA and CA group

The 16s rRNA sequencing data were analyzed with a focus on changes in bacterial relative abundance over time between the FA and CA group, was conducted. This analysis considered genera present with a relative abundance of at least 0.1% and in more than 25% of samples. This led to the identification of 48 distinct genera. However, changes in the relative abundance of these bacteria across different time points did not demonstrate any significant statistical difference between the FA and CA groups (data not shown).

The result of the microbiological analysis was then stratified to evaluate relationships between the relative abundance of different bacteria and either PI or GI score. Figure 11 compares the mean relative abundance of *Neisseria, Veilonella, and Capnocytophaga* with the plaque index score. When comparing patients with the lowest PI score of 1 a statistically significant higher level of *Neisseria and Capnocytophaga* were present in the CA group as compared to the FA group ($p<0.05$).

Patients from the FA group with a Plaque score of 3 had the highest mean relative abundance of *Neisseria* at 9.1%. There were no patients from the CA group with a PI as high as 3, and the relative abundance of Neisseria was lower for all other PI scores in both the CA and FA groups. *Veilonella* showed a similar trend of increase in its mean relative abundance with increase in plaque score for the FA group which was statistically significant as well ($p<0.05$).

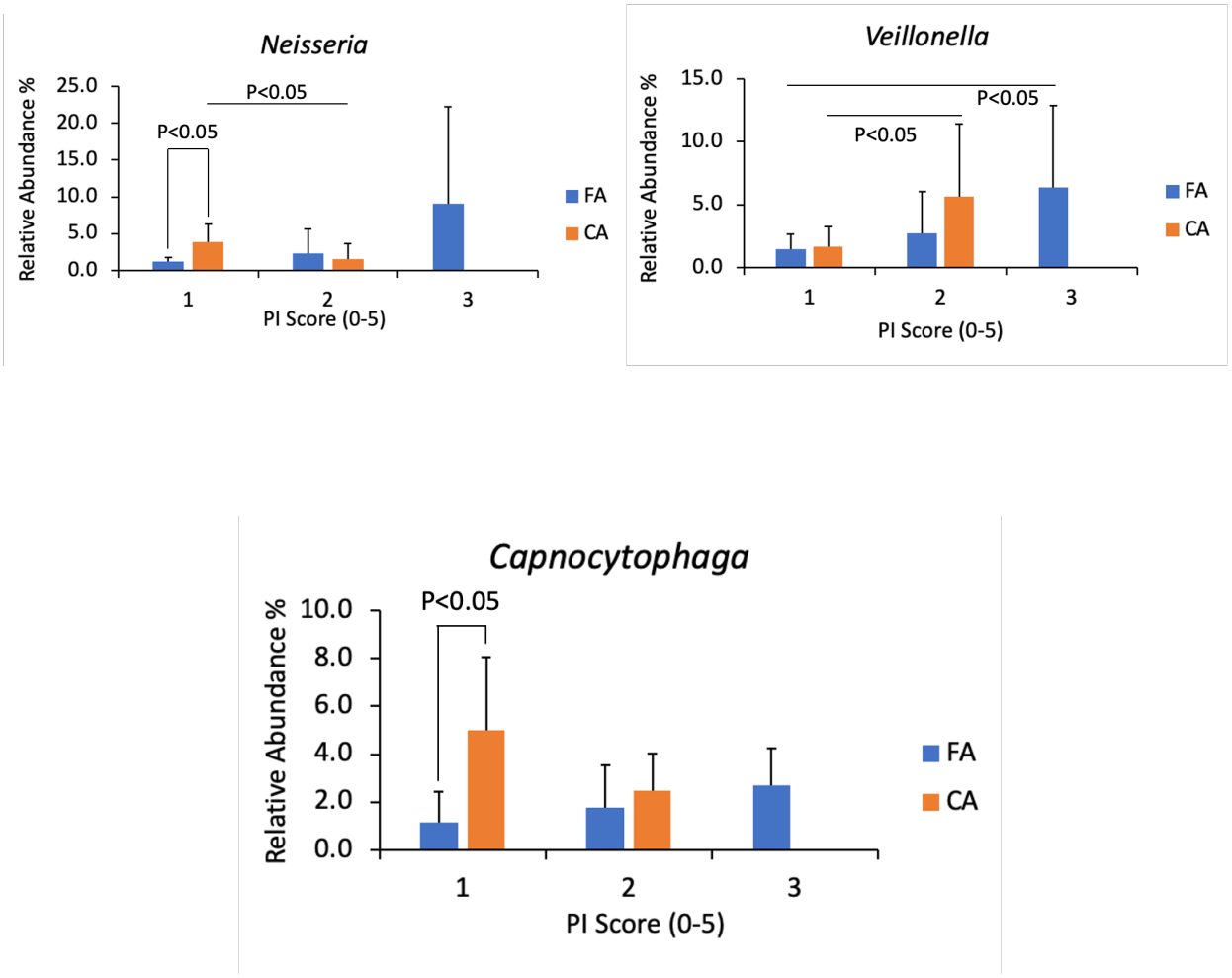


Figure 11: Comparing relative abundance of bacterial genera between FA (blue) and CA (orange) with the plaque index score.

Figure 12 compares the relative abundance of various bacterial genera with the gingival index scores. When comparing patients with the lowest GI score of 1 a statistically significant ($p<0.05$) higher relative abundance of *Neisseria* was found in the CA group relative to the FA group. Although not statistically significant, there was an increase in mean relative abundance of *Neisseria* for the FA group from 0.8 to 20.7 as the GI score increased from 1 to 3. In contrary, the relative abundance of *Leptotrichia* decreased when the GI score increased from 1 to 2 for the CA group. This was statistically significant at $p<0.01$. Although not statistically significant the relative abundance of *Leptotrichia* was lowest at high GI score for FA group.

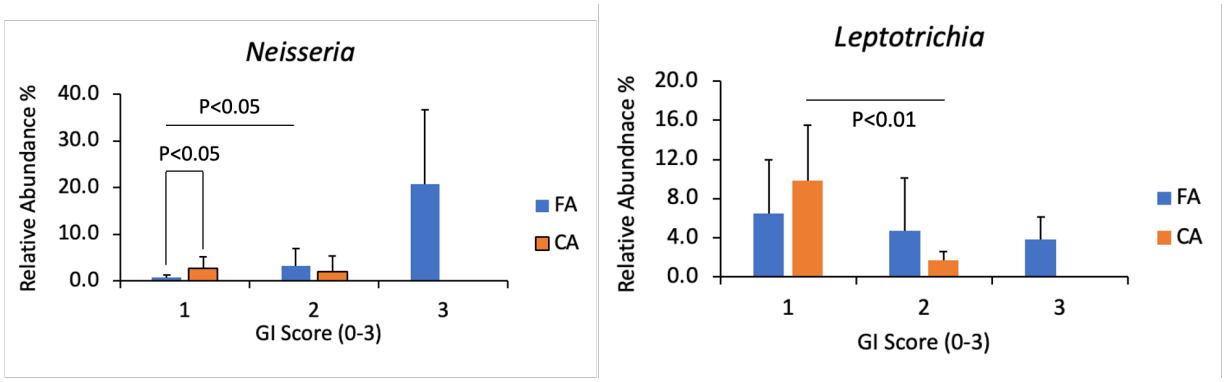


Figure 12: Comparing relative abundance of bacterial genera between FA (blue) and CA (orange) with the gingival index score.

The alpha diversity of the supragingival plaque were performed using Shannon Index in correlation with the gingival index, plaque index and two time points (T0 and T1). However, no correlation was found, and the results observed were found not to have a statistically significant difference (Figure 13).

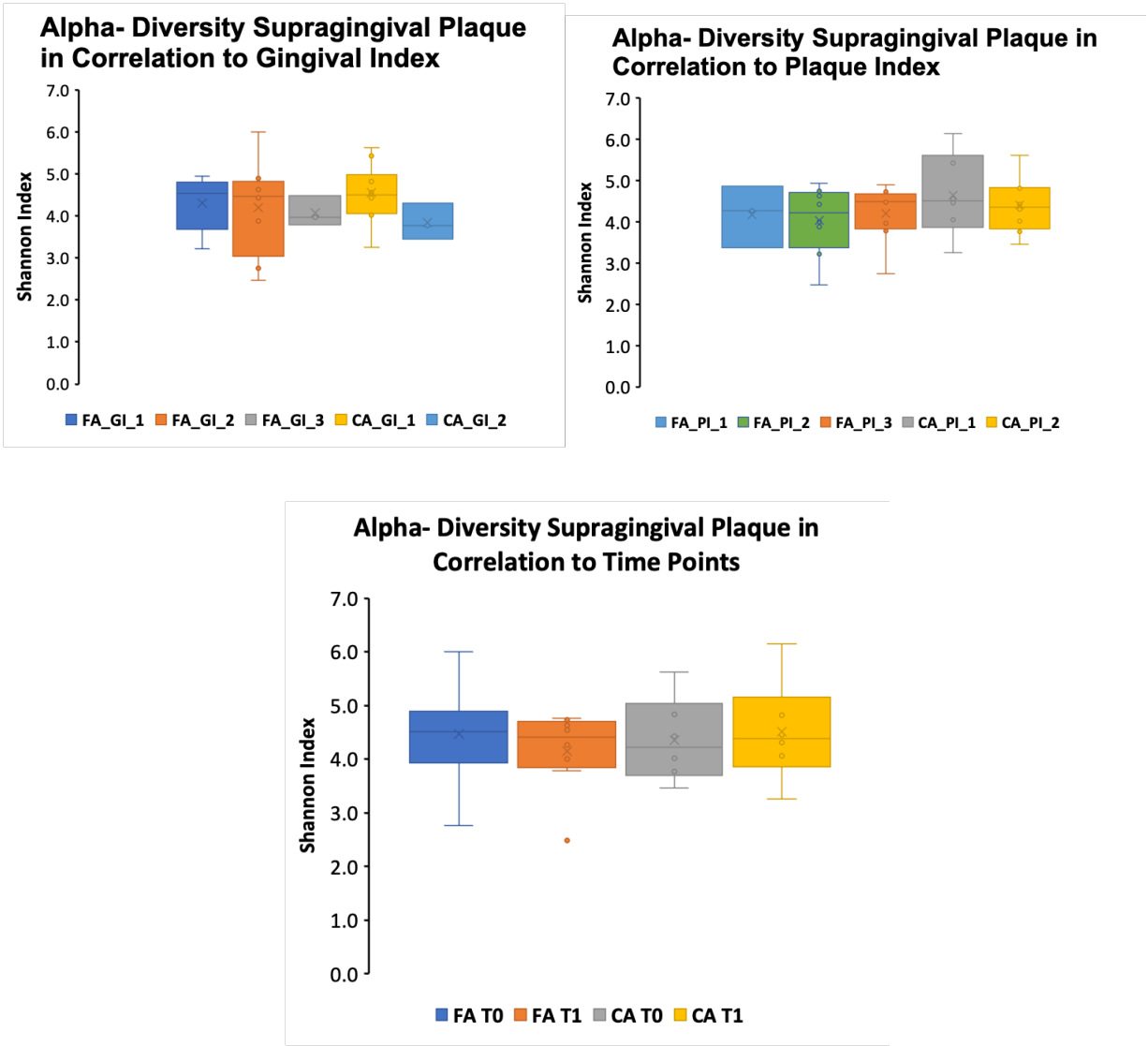


Figure 13: Alpha- diversity analysis. The top diagram shows the correlation with the gingival and plaque indices. The bottom diagram is separated based on time points (T0 and T1) in the FA and CA group.

Beta-diversity analyses via weighted UniFrac were performed on the samples, and the features driving the first two dimensions taken together explain more than 35% of the variation between samples. Figure 14 shows the conglomeration of all samples and reveals no clear distinction

between the treatment groups in relation to plaque index, gingival index, and different time points. The result was observed to have no statistically significant difference as well.

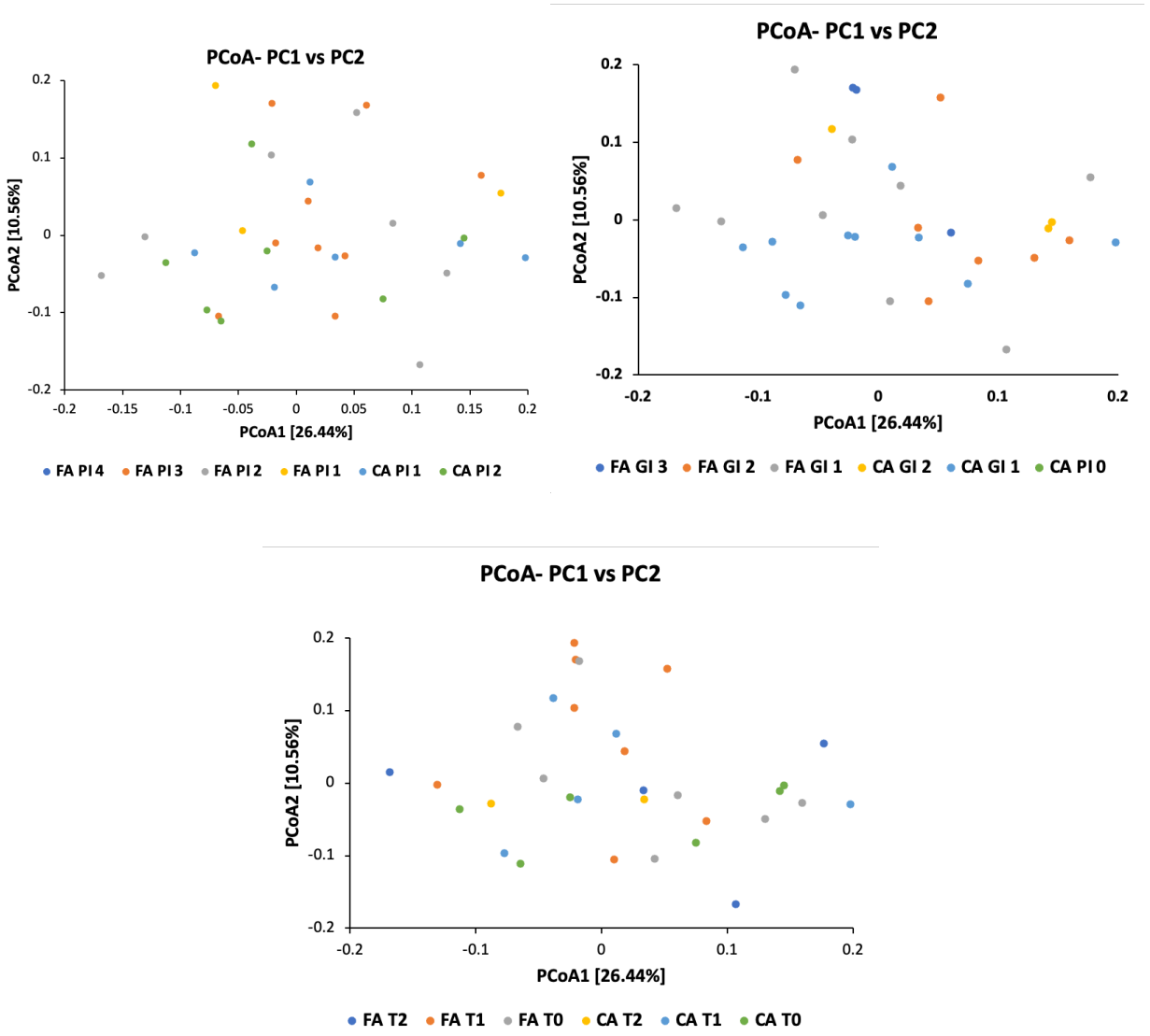


Figure 14: Beta- diversity analysis. The top diagram combines the FA and CA group with the plaque and gingival index and shows no clear distinction in both the correlation with plaque index and gingival index. The bottom diagram combines FA and CA group with the time points T0 and T1 and shows no distinction between FA and CA in those two time points.

DISCUSSION

The human oral cavity harbors a diverse microbiota exceeding 700 distinct species.[1] This microbial consortium's equilibrium plays a fundamental role in maintaining oral health and modulating host immune responses to pathogenic challenges. Any imbalance in this bacterial community can lead to substantial metabolic and immune system changes in our bodies.[2, 3] This could eventually pave the way for local and systemic diseases such as white spot lesions, caries, periodontitis, and metabolic disorders.[2, 40, 41] It's acknowledged that the introduction of fixed orthodontic devices and clear aligner therapy can lead to increased plaque and gingival indices, along with bacterial imbalance, following the commencement of orthodontic treatment.[10] In this study, our initial hypothesis suggested a decrease in plaque scores, gingival scores, and microbial abundance after debonding of braces.[27] However, existing research provides mixed results, with some studies reporting an increase in specific microbiota post-debonding, while others indicate a decrease.[24-26]

In a study by Shokeen et al. an increase in the PI scores after initiation of fixed aligner treatment was found.[42] Perhaps unsurprisingly then, in our research, we observed a trend of declining gingival and plaque index scores in both anterior and posterior sections of the teeth with increasing time after debonding up to the conclusion of the study (three months post-debonding). This was similar with the study conducted by Kyunsun Kim et al where they observed a decrease in GI and PI 1 week, 5 weeks, and 13 weeks after debonding.[26]

In our study the reduction in the gingival index and especially the plaque index was more pronounced in the Fixed Aligner (FA) group compared to the Clear Aligner (CA) group with

increasing time after debonding. This makes sense as patients with Fixed Aligners have been shown to have worse (higher) PI and GI scores relative to patients with Clear Aligners during orthodontic therapy.[42] This is likely due to the higher relative difficulty in maintaining good oral hygiene while having a fixed orthodontic appliance (FA) in one's mouth relative to a removable orthodontic appliance (CA). Similarly, our study found the FA group to have a statistically significant higher overall GI and PI score relative to the CA group at the time of debonding. It follows that the FA group experienced a larger drop in GI and PI scores compared to the CA group as there was more room for improvement in the oral hygiene of the FA patients relative to the CA patients once the orthodontic appliance was removed. The reduction in GI score was only slightly higher in the FA group than the CA group, and not by a statistically significant margin. However, the difference in the reduction for the plaque index was more substantial between the FA and CA groups. The plaque score reduction in the FA group was greater than the CA group by a statistically significant difference ($p<0.01$) between the day of debonding (T0) and three months after debonding (T3).

The microbial analysis results did not indicate a strong trend with respect to time for the relative abundance of the various bacterial genera. We analyzed bacteria that comprised more than 0.1% relative abundance in at least 25% of the samples, leading to an examination of 48 different bacterial genera. Across all genera, no statistically significant differences were consistently observed between the two types of appliances over time. This finding contradicts the study conducted by Woo Sung Jung et al.[24, 25] .Woo-Sun Junga et al also looked at salivary *Streptococcus mutans* levels 1 week, 5 weeks, and 13 weeks after debond and there was an

increase in *Streptococcus mutans* after debonding despite improvement in oral hygiene, which was not observed in our study, although we looked at the genus level of *Streptococcus* only.[25]

Cagri Turkoz et al in 2012 performed microbial analysis of plaque and saliva in patients wearing essix retainer after debond for up to 60 days.[43] According his study, there was an increase in levels of *S. mutans* and *Lactobacillus* levels. They concluded by saying that the essix retainer creates a plastic covering and can decrease salivary flushing, aiding in accumulation of plaque and increase in bacterial colonization. This can possibly explain the increase in mean relative abundance of few bacteria such as *Streptococcus* and *Lactobacillus* in my study after debonding for both FA and CA group [data not included since it wasn't statistically significant]. Patients in this study were also wearing an essix retainer for 1 month after debond. They were recommended to wear it all the time except while eating.

The FA group was associated with higher levels of various genera of bacteria known to be health associated such as *Neisseria, Veilonella*, and *Capnocytophaga*. *Neisseria* can be nonpathogenic commensal or pathogenic in the form of Neisseria gonnoreae.[44] *Veilonella* was studied and found in the tongues of healthy individuals however the pathogenicity of the bacterial species is not yet clarified.[45] *Capnocytophaga spp* are known to be less virulent but it is known to be associated with gingivitis and oral cancers especially in immunocompromised patients.[46, 47]

The CA group showed higher levels of *Neisseria*, *Leptotrichia* and *Capnocytophaga* at the lowest GI and PI score of 1. *Leptotrichia* was found to have increased in a study conducted by Shokeen at al after initiation of orthodontic therapy.[42] Studies show association of *Leptotrichia* with mucositis, oral lesions and wound abscess.[48]

Alpha and beta-diversity showed no significant differences in supragingival plaque when associated with PI, GI, or different time points for both the appliances.

The limitations of this work should also be noted. The relatively small sample size could obscure small to moderate correlations between the various variables investigated in this study, the power analysis being 40. Further, there was difficulty in collecting data at each time point for all patients due to the rescheduling of appointments. There was a much smaller amount of data collected at timepoint T2 (3 months after debond) with n=6 (FA=4, CA=2) despite having a total of 14 patients in the study. Moreover, the fact that the mean age of the CA group was approximately 5 years older than the FA group could also be considered a limitation of this investigation.

Additionally, this study only looked at supragingival plaque. Future work could be done to assess the microbiota in sub gingiva plaque and saliva for comparison. Finally, one-way, and two-way Analysis of Variance (ANOVA) can be done in the future to further enhance this study.

Supplemental Tables and Figures

Table S1: Mean Gingival and Plaque indexes taken at each time point for the fixed appliance and clear aligner groups.

Appliance Type	Time Point	n	GI (mean ± SD)	PI (mean ± SD)
Fixed Appliances	T0	12	2.09 ± 0.55	2.71 ± 0.94
	T1	12	1.52 ± 0.53	1.94 ± 0.65
	T2	6	1.28 ± 0.54	1.38 ± 0.66
Clear Aligners	T0	8	1.50 ± 0.35	1.77 ± 0.44
	T1	8	1.06 ± 0.53	1.25 ± 0.55
	T2	4	0.55 ± 0.18	0.65 ± 0.24

Sample Collection Data Sheet

Note: Do not include any patient identifying information

Subject ID#: _____

Date of visit: _____ Visit # *(Debond, 1 month, 3 month)*: _____

Did subject refrain from brushing teeth or eating? *(circle)* Yes No

Appliance Type *(Braces or Invisalign)*: _____

Retainer Type: _____

Samples ID#s:
Sample Label = [subject ID] [type of sample: S=saliva, A=Supragingival Plaque, B=Subgingival Plaque, T=Tray] [Visit #] *ex:* 1A3 (subject #1, supragingival plaque, visit #3)
Make sure matches label on collection tubes.

_____

_____

_____

_____

Notes:

Study: Changes in the oral microbiome in orthodontic patients after debond.

IRB#: 21-000340
PI: Renate Lux, 310-206-5660

Gingival Index

Score each tooth of their buccal, mesial, lingual, and distal surfaces of the gingival tissues by using Loe and Silness gingival index.

Scoring:

0 = Normal gingiva: Natural coral pink gingival w/ no e/o inflammation

1= Mild inflammation: Slight changes in color, slight edema. No bleeding on probing.

2 = Moderate inflammation: Redness, edema, and glazing. Bleeding on probing.

3 = Severe inflammation: Marked redness and edema/ulceration/tendency to bleed spontaneously

Codes:

X = tooth absent

B = bracketed tooth

O = banded

N = no bracket/band

A = attachment

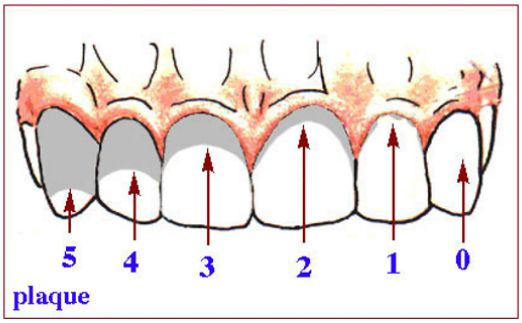
Upper tooth number	Code	Buccal surface score	Lingual surface score	Mesial surface score	Distal surface score
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					

Lower tooth number	Code	Buccal surface score	Lingual surface score	Mesial surface score	Distal surface score
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					

Plaque Index

Score **ALL** surfaces

Score	Criteria
0	No plaque
1	Separate flecks of plaque at the cervical margin of the tooth
2	≤1mm continues band of plaque at the cervical margin of the tooth
3	A band of plaque >1mm but <1/3 the crown of the tooth
4	Plaque covering 1/3 – 2/3 the crown of the tooth
5	Plaque covering >2/3 the crown of the tooth



Tooth number	Buccal Score	Lingual Score	Tooth number	Buccal Score	Lingual Score
2			18		
3			19		
4			20		
5			21		
6			22		
7			23		
8			24		
9			25		
10			26		
11			27		
12			28		
13			29		
14			30		
15			31		
Sum			**Sum**		

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