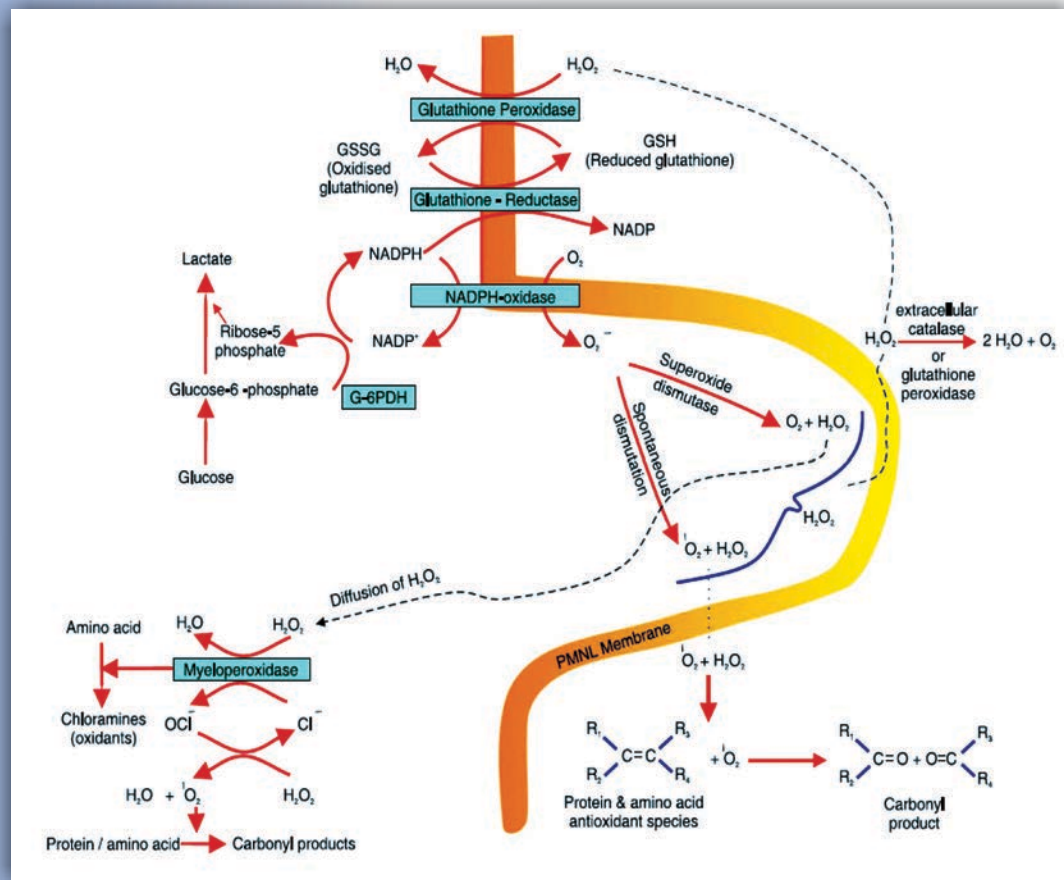


EJD EUROPEAN JOURNAL of DENTISTRY

The official journal of Dental Investigations Society

Volume 9 Number 3 Jul - Sep 2015



Periodontal health status in patients treated with the Invisalign® system and fixed orthodontic appliances: A 3 months clinical and microbiological evaluation

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ABSTRACT

Objective: The aim of this prospective study was to compare the periodontal health and the microbiological changes via real-time polymerase chain reaction (PCR) in patients treated with fixed orthodontic appliances and Invisalign® system (Align Technology, Santa Clara, California). **Materials and Methods:** Seventy-seven patients were enrolled in this study and divided into three groups (Invisalign® group, fixed orthodontic appliances group and control group). Plaque index, probing depth, bleeding on probing were assessed. Total biofilm mass and periodontal pathogens were analyzed and detected via real-time PCR. All these data were analyzed at the T0 (beginning of the treatment) T1 (1-month) and T2 (3 months); and statistically compared using the Mann–Whitney test for independent groups. **Results:** After 1-month and after 3 months of treatment there was only one sample with periodontopathic anaerobes found in patient treated using fixed orthodontic appliances. The Invisalign® group showed better results in terms of periodontal health and total biofilm mass compared to the fixed orthodontic appliance group. A statistical significant difference ($P < 0.05$) at the T2 in the total biofilm mass was found between the two groups. **Conclusion:** Patients undergoing orthodontic treatment with the Invisalign® System show a superior periodontal health in the short-term when compared to patients in treatment with fixed orthodontic appliances. Invisalign® should be considered as a first treatment option in patients with risk of developing periodontal disease.

Key words: Clear aligners, fixed orthodontic treatment, Invisalign, microbiological evaluation, periodontal health

INTRODUCTION

Treatment with fixed orthodontic devices such as brackets and bands creates numerous plaque accumulation sites impeding oral hygiene procedures and thus potentially leading to develop white spot lesions, caries, and periodontitis.^[1,2]

It is recognized that microbial dental plaque is the main etiologic factor in the development of dental caries and periodontal disease.^[3]

Plaque accumulation can favor the transition of the microbial biofilm to a more aggressive periodontopathogenic flora in subgingival periodontal pockets and the production of proinflammatory cytokines.^[4,5]

During fixed orthodontic treatment inflammation occurs and pathologic phenomena such as gingivitis, gingival bleeding, gingival enlargement, and increased gingival pocket depth are observed.^[6]

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How to cite this article: Levrini L, Mangano A, Montanari P, Margherini S, Caprioglio A, Abbate GM. Periodontal health status in patients treated with the Invisalign® system and fixed orthodontic appliances: A 3 months clinical and microbiological evaluation. Eur J Dent 2015;9:404-10.

DOI: 10.4103/1305-7456.163218

Furthermore, microbiological studies revealed significant changes in the bacterial composition of the subgingival dental plaque, so orthodontic treatment may affect the equilibrium of oral microflora and increase bacteria retention.^[7] It has been shown that treatment with fixed orthodontic appliances stimulates the growth of a subgingival plaque where some periodontopathogenic bacterial strains are prevalent such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Treponema denticola*.^[8-10]

In contrast, the use of removable orthodontic appliance is able to allow an adequate oral hygiene and reduce the risk for such negative dental and periodontal complication.^[11,12] Orthodontic treatment should be able to expose the patients to none or limited side effects. Along with the risk of root resorption periodontal complications are the most reported to occur. Periodontal health should be regarded as one of the success criteria in orthodontic treatment.^[13]

In 1999, a new orthodontic system based on a polymer composed by a chain of organic units joined with urethane links was introduced (Invisalign®, Align Technology, Santa Clara, California) as a removable appliance able to gradually move the teeth to a treatment plan, which was formerly computer designed.^[14]

Few studies evaluated the subgingival pathogenic microflora via real-time polymerase chain reaction (PCR) analyses in fixed orthodontic therapy,^[15-18] and there is only one preliminary report evaluating microbiological and periodontal data related to periodontal disease risk development in patient treated with fixed and removable appliances.^[19]

To date, the literature is scarce about scientific trials that are committed to elucidate the relationship between orthodontic treatment and periodontal health status.^[20] To the authors' knowledge, there are only clinical trials evaluating the periodontal health of clear aligners compared to traditional orthodontic appliances^[11,12] but none compared the microbiological aspect of the Invisalign® treatment to the fixed orthodontic appliances.

The hypothesis that we investigated was that patients treated with Invisalign® aligners had a better periodontal health compared to patients who were treated with fixed appliances.

Thus, the aim of this study was to evaluate the total microbiological biofilm mass and the presence

of selected bacteria, via real-time PCR, in adults undergoing fixed or removable orthodontic therapy with the Invisalign® system. The modified plaque index (PI), pocket probing depth (PD), and the bleeding on probing (BOP) were also evaluated during the entire period of treatment by clinical assessment.

MATERIALS AND METHODS

Patient population

Seventy-seven patients, 52 females and 25 males with a mean age of 24.3 years (range from 16 to 30) were included in this study referring to the Department of Orthodontics of the University of Insubria.

Sixty-seven patients referred to our clinic for orthodontic treatment and were randomly selected to the test Invisalign treatment group and the fixed appliance treatment group. A group of ten patients who did not need any treatment was used as control group. The composition of the three groups is shown in Table 1.

Exclusion criteria

- Smoking habit
- Presence of extensive dental restorations in proximity to the gingival margin
- Presence of fixed bridges/crowns or partial dentures
- Previous periodontal nonsurgical treatment (such as full mouth disinfection, quadrant-by-quadrant therapy, full mouth debridement) within the past year
- Medications such as antibiotics, steroids, or nonsteroidal anti-inflammatory drugs within the past 6 months.

Moreover, the patients used no oral antiseptic solutions or mouthwash during the entire investigation, but who used dietary supplements with antioxidant properties were not excluded.

Inclusion criteria

- Class I skeletal relationship
- Normo-divergent Frankfort Mandibular-Plane Angle
- Age >16

Table 1: Composition of the groups

Group	Male	Female	Total
Invisalign	5	27	32
Fixed orthodontics	18	17	35
Control	2	8	10
			77

- Class I molar relationship
- Minimal mandibular crowding in a range from 1 to 3 according to Little's Index.^[20]

All patients were informed of the nature of the study to be carried out on an individual basis and read and signed a written consent form. The study protocol was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2007.

One-month before orthodontic therapy, professional oral hygiene was performed, and patients were instructed on a standardized oral hygiene protocol. Oral hygiene instructions were specified by an experienced dental hygienist before the treatment and recapitulated during all the scheduled check-up. Electric toothbrushes were not allowed in the protocol. All patients had to use an orthodontic brush (bass technique for 2 min) and dental floss three times a day.

The fixed orthodontic treatment was performed in all patients by treating the upper and lower arch simultaneously. Mini Sprint brackets (Forestadent®, Pforzheim, Germany) and standard elastic ligatures were used on incisors, canines, and premolars; orthodontic bonded tubes were used for the first molars (Forestadent®). The bonding procedure was performed with a direct technique using Transbond XT (3M, St. Paul, MN, USA). The patients in the Invisalign® group were instructed to wear the aligners 20 h a day. The Invisalign® aligners were replaced every 2 weeks with a new set, which had been previously developed according to the treatment plan of each single patient.

Periodontal indices

The clinical assessment of the periodontal health status was achieved using the periodontal index, according to the criteria of the modified PI of Loe and Silness,^[22] pocket PD and BOP.^[5,21] The pocket PD was measured to the nearest millimeter on the scale of the periodontal probe (Goldman-Fox, Hu-Friedy Mfg Co., Inc., Chicago, IL, USA) and BOP tendency was registered 20 s after probing (absent = 0, present = 1).^[5] The PI was assessed by observing the plaque accumulation in the gingival area and was classified into one of four grades.^[21] Scoring criteria were:

- 0 = No plaque/debris on inspection and probing
- 1 = Thin film of plaque only visible after probing
- 2 = Ribbon-like layer of plaque covering the gingival sulcus with no involvement of interproximal dental space
- 3 = Thick layer of plaque clearly visible at inspection and involving an interproximal dental space.

These clinical parameters were assessed on the mesio-vestibular surface of the examined teeth: Upper right first molar (Site 0) and upper left central incisor (Site 1), according to the Ramfjord system.^[22] This periodontal assessment was performed at the beginning of the orthodontic treatment (T0), after 1-month (T1) and after 3 months, corresponding to the end of the treatment (T2). The scoring registrations were executed by a single calibrated examiner while all reviews according to the protocol were carried out by two operators who were unaware of the experimental protocol.^[23]

Evaluation of total biofilm mass and periodontopathic bacterial species

The microbiological samples were obtained from the same sites (Site 0 and 1) at T0, T1, and T3 as previously described in the periodontal assessment. In order to evaluate the biofilm present in the experimental sites, the microbiological investigation was performed to confirm the presence or absence of four periodontopathic anaerobes species: *P. intermedia*, *A. actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythia*. These samples were collected in dry field conditions by inserting one sterile paper point into the deepest part of the gingival sulcus for 30 s.^[24] After insertion, paper points were closed into a test tube, refrigerated at -20°C and sent to the DNA sequencing service, University of Cagliari, Italy, where the microbiological analysis was performed. Periodontal pathogens and total biofilm mass were detected by real-time PCR procedures.^[25,26]

Molecular analysis

Each paper point was suspended in 50 µl of pure dimethyl sulfoxide (DMSO) and centrifuged for 30 s. Two microliter was used as DNA suspension for real-time PCR reactions. Periodontal pathogen and total bacteria enumeration (biofilm mass) were detected by real-time PCR procedure and molecular analysis protocols used in this paper has been described in previously published papers.^[24,25]

The periodontal bacteria quantification was performed using the oligonucleotides described for conventional PCR. Real-time PCR was performed using a LightCycler instrument and a LightCycler DNA Master SYBR Green I kit (Roche Diagnostics Mannheim Germany), according to the manufacturer's instructions. Among 10 fold serial dilutions of each bacterium in DMSO ranging from 10⁷ to 10² cells/ml was prepared. These suspensions served as a standard curve for measuring the pathogen concentration. PCR mixture

contained (20 µl final volume): 4 mM MgCl₂, 1 µM of each primer, and 2 µl of DMSO suspension. The PCR program was the following: (i) Denaturation at 95°C for 30 s, (ii) 40 cycles of 10 s at 95°C, 10 s at 50°C, 12 s at 72°C, (iii) melting curve performed for 10 s at 95°C, 45°C, 95°C. Transition rates were 5°C/s in the 72°C segment, 0.1°C/s in the 45°C segment, and 20°C/s for another step. Fluorescence was detected at the end of the 72°C segment in the PCR step (single mode), and at the 45°C segment in the melting step (continuous mode) in the F1 channel. During initial optimization of real-time reaction, PCR products were analyzed using agarose gel and by a melting curve analysis to ensure correct sample product size. The positive reactions showed 7–90°C T_m peaks. The amount of bacterial DNA in the samples was calculated following sequent formula ($C = q \times 25$), C is the final bacterial concentration (totals or single periodontal pathogen) in the specimen; q is the bacterial number calculated interpolating threshold cycle with a qPCR standard curve.

Statistical analysis

To compare the differences of the periodontal indices such as PI, BOP, PD; and the differences between the microbiological biofilm in the patients treated respectively with Invisalign®, fixed appliances and control group the Mann-Whitney test for independent groups was performed. The level of significance was set at 0.05. Furthermore, the Mann-Whitney test was performed to test differences between different time-points in each group. The level of significance was set at 0.05. All statistical analyses were run on the statistical package SPSS (SPSS 17.0; SPSS Inc., Chicago, IL, USA). A priori sample size calculation was performed with $\alpha = 0.05$ and a power set at 80%.

RESULTS

The microbiological analyses detected the presence of *A. actinomycetemcomitans* only in one patient treated with fixed orthodontic appliances at T1 and T2.

A statistically significant difference ($P < 0.05$) was found between the Invisalign® group and the fixed orthodontic appliance group in all periodontal parameters (BOP, PD, and PI) with the Invisalign® group scoring lower values compared to the fixed orthodontic appliance group. Furthermore, the total biofilm mass showed a statistically significant differences ($P < 0.05$) between the Invisalign® group

and the fixed orthodontic appliance group. The periodontal parameters showed worst scores in T2 compared to T0 and T1 in the fixed orthodontic appliance group, as well as the total biofilm mass. The Invisalign® group showed a statistically significant increase in the PI values in the T2 compared to T0. Furthermore, no statistically significant differences in the BOP and in the PD were observed.

The Invisalign® group showed a statistically significant difference ($P < 0.05$) between the T2 and T0 in the total biofilm mass with a lower score in the 90 days follow-up control. In the control group, no statistical differences were found between the follow-up controls.

At T2 the mean bacterial concentration “C” was 104,536,026; 2739 and 8187 in the fixed orthodontic, the Invisalign® and control group, respectively, being significantly lower for the two latter groups. The mean PD in the fixed orthodontic group; Invisalign® group and control group at T2 were, respectively, 1.3;1.6;1.7. At T1 the mean PD values were 2.20; 2.75; 2.15. At T0 the mean PD values were 2.18; 2.18; 2.15. At the beginning of the study the mean Little’s Index score for the Invisalign® group was 2.3 ± 0.3 , for the fixed appliances group 2.5 ± 0.4 and for the control group 2.3 ± 0.4 .

DISCUSSION

The present study has described microbiological and periodontal changes in two groups of patients treated, respectively, with fixed appliances and Invisalign® removable aligners. The effect of orthodontic appliances on periodontal health has been evaluated in many studies.^[2,5-8]

A systematic review of the literature about the relationship between orthodontic treatment effect and periodontal health stated that gingivitis and attachment loss were inconsistent across studies, and that there is an absence of evidence supporting positive effects of orthodontic treatment on overall periodontal health, but many data indicate that orthodontic therapy may result in small detrimental effects to the periodontium.^[1]

Plaque accumulation is the main etiological factor, and gingival inflammation enhances the flowing of gingival crevicular fluids that supply plasma proteins, which are essential for the growth of proteolytic anaerobes.^[28]

Our data showed how fixed orthodontic treatment group resulted in higher plaque accumulation compared to Invisalign® treatment group. This data are in accordance with our previous study.^[19] This result can be attributable to easier oral hygiene procedures favored by a better accessibility in Invisalign® patients. Furthermore, fixed orthodontic devices present more plaque retention sites that potentially lead to periodontal inflammation.^[18]

A significant increase of PI and BOP was found in patients treated with fixed orthodontic, but PD index had no significant changes. The increase in biofilm mass was a direct consequence of impeded oral hygiene procedures. This clearly show how patients treated with fixed appliances are more likely susceptible to gingival inflammation that eventually could develop to periodontal disease.^[27]

A recent investigation by Ghijssels *et al.*^[30] showed how in the long-term patient treated with fixed orthodontic appliances have a worsening in the periodontal parameters. On the contrary, the microbiological analyses underlined a significantly difference between the aerobe/anaerobe ratio prior and after the treatment.^[30] Some clinical studies reported poor periodontal health and greater loss of clinical attachment level in the distal area of the dental arches in patients treated with fixed orthodontic treatment. These worse data could be the result of poor oral hygiene in molar regions also due to the presence of molar bands, which favor food entrapment.^[31]

Statistical differences between T0 and T2 of the BOP, PD and PI were found in the fixed orthodontic treatment group. These results are similar to the one reported by Ristic *et al.*^[29] and by Demling *et al.*^[32] that observed an increase in the periodontal indices and a modification of the microbiological composition. The change in the microbiological composition with the shift of microbiological flora is due to the food entrapment that eventually lead to plaque accumulation and inflammation.^[29] In our study, we performed a professional cleaning in order to eradicate possibly periodontal pathogens. A recent investigation using atomic force microscope by Germano *et al.*^[33] showed how periodontitis bacteria have complex glycocalyx being also able to co-aggregate thus improving their resistance to antibiotics.

Decreased plaque level were found in the Invisalign® treatment group and were associated with better periodontal health indices; these results are in accordance

with Karkhanечи *et al.*^[34] A possible explanation can be attributable to easier oral hygiene procedures; the absence of bands, brackets and archwires in the patients treated with Invisalign® can favor the maintenance of better oral hygiene.^[19] A recent systematic review of the literature^[35] showed how clear aligner treatments have an improvement in periodontal health indexes compared to fixed orthodontic treatments.

The overall higher periodontal indices and microbiological results can be attributable to better compliance in oral hygiene procedures in Invisalign® group as showed in a previous study. Our results have been confirmed by a recent study.^[35] In this study, a better compliance to oral hygiene procedures in patients treated with Invisalign was observed. A main difference in our study, is that we did not evaluate the patients' compliance. As the clear aligners are removable appliances giving to the patients easy access to all teeth surfaces it can be assumed that this treatment option should be a first choice in adult patients and in patients with possible periodontal problems.

It is important to stress that a careful hygiene maintenance of the aligners must be performed in order to control the plaque accumulation on the clear aligners.^[36]

One of the limitations of this study, is the short follow-up period, a longer observational time would be useful for the evaluation of the plaque accumulation and the periodontal indices because the change of the bacterial flora in patients receiving fixed appliances take place in the first 3 months.^[8,37,38]

To our knowledge, this is the first prospective study, to compare the periodontal status and the plaque accumulation via real-time PCR between fixed buccal appliances and removable aligners in the short-term period. Although periodontal status showed the worst score in patients receiving the fixed appliance treatment, we do not suggest to avoid this kind of treatment in adult patients. In fact, not all treatment objectives can be achieved with clear aligners. Furthermore, there is still uncertainty about the long-term possible negative effect of fixed orthodontic appliance on periodontal health.

CONCLUSIONS

Within the limit of this study, we can state that:

- Patients treated with removable aligners had a

better periodontal health status (PI, PD, BOP) compared to patients treated with fixed appliances

- Removable aligners seem to facilitate oral hygiene procedures
- Absence of periodontal pathogenic bacteria in Invisalign® treatment group
- Real-time PCR analysis detected a periodontopathic bacteria in one patient treated with fixed orthodontic device
- Real-time PCR showed higher level of bacteria concentration in patients treated with fixed orthodontic device.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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