
Assessment of the periodontal health status in patients undergoing orthodontic treatment with fixed or removable appliances. A microbiological and preliminary clinical study

Luca Levrini, DDS, PhD,^a Gian Marco Abbate, DDS,^b Federico Migliori, DDS,^c Germano Orrù, DDS, PhD,^d Salvatore Sauro, PhD,^e Alberto Caprioglio, DDS,^f

^aFaculty of Dentistry, University of Insubria, Varese, Italy.

^bDepartment of Dental Hygiene, School of Dentistry, University of Insubria, Varese, Italy.

^cDepartment of Orthodontics, School of Dentistry, University of Insubria, Varese, Italy.

^dDepartment of Surgery and Odontostomatological Sciences, O.B.L., University of Cagliari, Cagliari, Italy.

^eDental Biomaterials Science, Biomaterials Biomimetics and Biophotonics group (B³), King s College London Dental Institute at Guy s, St Thomas Hospitals, London, UK. B); Universidad CEU-Cardenal Herrera - Facultad de Ciencias de la Salud, Department of Dental Biomaterials and Biomedical Sciences, Alfara del Patriarca-Valencia, Spain.

^fDepartment of Orthodontics, School of Dentistry, University of Insubria, Varese, Italy.

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ABSTRACT

Objectives: The use of removable orthodontic appliances minimizes the negative effects on periodontal health allowing patients to carry out oral hygiene without obstacles. The aim of this preliminary study was to evaluate microbiological and clinical changes presented during the first three months of orthodontic therapy in adults with fixed appliances and Invisalign® System (Align Technology, Santa Clara, California).

Materials and Methods: Plaque Index (PI), Probing Depth (PD), Bleeding on probing (BOP), Compliance to oral hygiene and subgingival microbial samples were assessed in 30 patients. Samples were analyzed by real time PCR to detect periodontal pathogens and microbial biofilm mass. A statistical comparison was made over time and amongst the three groups, using Chi-square X², Odds Ratios (OR), Regression analysis (DOE) and ANOVA.

Results: After 30 and 90 days of treatment there was only one sample with periodontopathic anaerobes found in a patient treated using fixed orthodontic appliances. Direct influence of orthodontic treatment on compliance and less subgingival biofilm mass were found with Invisalign® patients who increased the time dedicated to oral hygiene. A decrease on PD (p=0,002) and BOP (p<0,001) was detected in the Invisalign® group after 90 days of treatment.

Conclusions: In this preliminary study, fixed and removable appliances did not increase the risk for periodontal disease in patients undergoing orthodontic therapy. However, the removable Invisalign® appliances may facilitate oral hygiene procedures, maintaining a lower level of microbial biofilm mass, even with poor oral hygiene compliance, minimizing the negative effects on gingival inflammation.

Keywords: Orthodontic treatment, Invisalign® system, fixed appliances, periodontal health, real-time PCR, oral hygiene compliance.

Gian Marco ABBATE,
Via C. Battisti
3- Castiglione Olona (Va)
21043-Italy
Tel: 0039 0331824975
e-mail: gmabbate@me.com

INTRODUCTION

Orthodontic therapy may favour an unpredicted accumulation of bacterial plaque on the dental surfaces in particular when fixed appliances are employed during the treatment. Several studies have

demonstrated how an excessive accumulation of bacterial biofilms in correspondence of fixed orthodontic appliances might cause significant enamel demineralization,¹ gingival inflammation,^{2,3} and an increase in probing depth.^{4,5,6} Furthermore, it has been hypothesized that an abnormal growth of the bacterial plaque in patients undergoing fixed orthodontic therapy may also have negative effects on the periodontium and trigger the development of the periodontal disease.¹ Socransky and Haffajee⁷ showed that the presence of fixed orthodontic appliances encouraged the growth of periodontopathic bacteria species such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* and *Treponema denticola*. Nevertheless, the use of alternative removable orthodontic appliances may allow patients to maintain an adequate oral hygiene and reduce the risk for such negative dental and periodontal complication.^{8,9,10} A new orthodontic system based on a polymer composed by a chain of organic units joined with urethane links has been recently 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.¹¹ Although the periodontal health in patients undergoing this type of orthodontic treatment has been already evaluated based on the assessment of the modified Plaque Index (MPI), Papillary Bleeding Index (PBI) and Probing Depth (PD),^{12,13} there is little information on the microbiological evaluation of the subgingival pathogenic microflora *via* real-time PCR analyses which may be a suitable method to estimate the risk for periodontal disease.^{14,15,16,17}

Therefore, this study aimed to evaluate the total microbiological biofilm mass and the presence of *selected bacteria* in adults

undergoing fixed or removable orthodontic therapy with the Invisalign® System. The oral hygiene compliance, 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. The null hypotheses to be tested were: 1) the different types of orthodontic appliances (fixed or removable) produced no increase of the risk for periodontal disease; 2) the removable Invisalign® System offered no benefits to the bacterial plaque control (Patients' oral hygiene compliance) and to the periodontal health status (periodontal indices) when compared to fixed orthodontic appliances.

MATERIALS AND METHODS

Criteria for patient recruitment and orthodontic procedures

Thirty adult patients (9 males, 21 females, aged 25.1 ± 4.6) attending the Department of Orthodontics, School of Dentistry, University of Insubria, Varese, Italy were selected for this study. The present study was conducted in accordance with the Helsinki Declaration and informed consent was obtained from all participants before the experiments. Exclusion criteria included smokers; presence of extensive dental restorations in proximity to the gingival margin; presence of fixed bridges/crowns or partial dentures; previous periodontal treatment within the past year; medications such as antibiotics, steroids, or non-steroidal anti-inflammatory drugs within the past 6 months. The patients used no oral antiseptic solutions or mouthwash during the entire investigation, but who used dietary supplements with anti-oxidant properties were not excluded. In order to have a homogeneous sample, subjects with Class I skeletal relationship, normo-divergent, Class I molar relationship and with minimal irregularity in a range of mandibular crowding from 1 to 3, according to Little's Index were selected.¹⁸

The mean Little's Index score for the Invisalign group was 2.5 ± 0.4 , for the fixed appliances group 2.6 ± 0.3 and for the control group 2.3 ± 0.4 .

All patients were informed of the nature of the study to be carried out on an individual basis and an informed consent was obtained. 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 re-capitulated during all the scheduled check-ups. Electric toothbrushes were not allowed in the protocol. All patients had to use an orthodontic brush (Bass technique for two minutes) and dental floss three times a day.

The 30 subjects selected were randomly assigned one of the three groups: A) 10 subjects were treated with Invisalign® aligners (Align Technology, Santa Clara, California); B) 10 subjects were treated with a traditional fixed orthodontic appliance; C) 10 subjects were assigned to a control group and received no orthodontic treatment. In order to have baseline equipoise for PI, PD and BOP between the groups, professional oral hygiene was performed to all patients one month before orthodontic therapy. Randomization was stratified based on age and gender. Each group had 3 males and 7 females, with a mean age of 24.6 ± 6.4 for patients treated with Invisalign, 25.7 ± 3.4 with fixed appliances and 25.0 ± 3.4 for the control group. The fixed orthodontic treatment was performed in all patients by treating the upper and lower arch simultaneously. Mini Sprint brackets (Forestadental®, Pforzheim, Germany) and standard elastic ligatures were used on incisors, canines and premolars; orthodontic bonded tubes were used for the first molars (Forestadental®). The bonding procedure was performed with a direct technique using Transbond XT (3M, St.

Paul, MN, United States). The patients in group (A) were instructed to wear Invisalign® aligners 20 hours a day. The Invisalign® aligners were replaced every two weeks with a new set which had been previously developed according to the treatment plan of each single patient.

Assessment of the hygiene compliance and periodontal indices

Oral hygiene compliance was assessed in each treatment group at the beginning of the study (T0), after 30 days (T1) and 90 days (T2) using the following scoring criteria:

(0) for all the patients at time 0 of the treatment;

(1) patients showing no improvement in oral hygiene compliance,

(2) patients showing a slightly increased oral hygiene compliance;

(3) patients showing a significant improvement in oral hygiene compliance.

This clinical protocol was performed 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 and of the clinical status of the patients throughout the study. The operators were previously calibrated using standardized parameters before the beginning of the study.

The clinical assessment of the periodontal health status was achieved using the periodontal index according to the criteria of the modified Plaque Index (PI) of Löe & Silness, pocket probing depth (PD) and bleeding on probing (BOP).^{5,19} The pocket probing depth (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 bleeding on probing (BOP) tendency was registered 20 seconds after probing (absent=0, present=1).⁵ The Plaque Index (PI) was assessed by observing the plaque accumulation in the gingival area and was

classified in one of four grades.¹⁹ 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.²⁰ 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.²¹

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 quantitatively evaluate the biofilm present in the experimental sites (microbial biofilm mass), the microbiological investigation was performed to confirm the presence or absence of four periodontopathic anaerobes species: *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas 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 seconds.^{22,23} After insertion, paper points were closed into a test tube, refrigerated at -20° C and sent to the DSS (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.^{24,25}

Molecular analysis

Each paper point was suspended in 50 µl of pure dimethyl sulfoxide (DMSO) and centrifuged for 30 seconds. 2 µl of this 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 basis 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. 10 fold serial dilutions of each bacteria in DMSO ranging from 10⁷ -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 sec, (ii) 40 cycles of 0 sec at 95 °C, 10 sec at 50 °C, 12 sec at 72°C, (iii) melting curve performed for 0 seconds 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 [$C = q \cdot 25$], C is the final bacterial concentration (totals o single pathogen) in the specimen, q is bacterial number calculated interpolating threshold cycle with a qPCR standard curve.

Statistical analysis

The microbiological results were statistically analyzed and compared with six independent variables: 1) Treatment; 2) Site; 3) Time; 3) Plaque Index; 4) Bleeding on probing; 5) Probing depth; 6) Oral hygiene Compliance. The dependent variable was the biofilm concentration. Groups did not differ at baseline in PI, PD, BOP and total biofilm mass. The comparison of the variables between T0, T1 and T2, for each treatment group, was made by Student's t-test (unpaired). The differences in bacteria concentration frequencies between cases and controls were determined using the Chi-square (χ^2) test. The relation between bacteria concentration and variables were analyzed by calculating the crude Odds Ratios (OR) and 95% Confidence Intervals (CI). Mallows' Cp was used to identify the best study model. Analyses of the correlation, Regression analysis (DOE) and Analysis of variance (ANOVA) were performed to evaluate the variables. Statistical significance was set at $p = 0.05$, data were analyzed with statistical software (Minitab®, version 15.1.1.0 for Windows, Minitab Inc, State College, Pennsylvania).

RESULTS

The microbiological investigation showed the presence of *Aggregatibacter actinomycetemcomitans* only in one patient treated with fixed orthodontic appliances at T1 and T2. The analyses of the correlation showed a statistical relation between the increase of microbial biofilm mass and the type of orthodontic treatment (OR: 0.65/95%; 0.48-0.89, $p < 0.005$), increase of Plaque Index (PI) (OR: 0.09/95% CI: 0.05-0.15, $p < 0.001$), bleeding on probing

(BOP) (OR: 0.20/95% CI: 0.11-0.36, $p < 0.001$) and a strong inverse relation with the patient compliance to oral hygiene (OR: 2.17/95% CI: 1.66-2.83, $p < 0.001$). A decrease of PD ($p = 0.002$) and BOP ($p < 0.001$) was detected in the Invisalign® (Align Technology, Santa Clara, California) group between T1 and T2 (Table 1). The patients treated with fixed orthodontic appliances showed a higher value of both PD and BOP at T2 in comparison to that found during the first appointment after 30 days (T1). Mallows' Cp was used to identify the best study model and the parameters suitable for the analysis of the biofilm mass results. The most significant variables showed by the test were Treatment, Plaque Index (PI) and Compliance. The Regression analysis (DOE) performed on the biofilm mass result showed a strong influence of both PI ($p < 0.001$) and Compliance ($p < 0.001$). Analysis of variance (ANOVA) showed that the total biofilm mass results obtained in both the orthodontic treatments were strongly influenced by Plaque Index (PI). However, when the microbial biofilm mass obtained in the three different treatment groups were compared at the same PI value (Figure 1), it was possible to observe that the removable treatment performed with the Invisalign® System induced a lower total biofilm mass accumulation (PI/T0; PI/T1; PI/T2) compared to the treatment performed with fixed appliances. The graphic analysis of the interaction between oral hygiene compliance and orthodontic treatments (Figures 2 and 3) showed that the fixed appliances had a negative significant influence on the final microbial biofilm mass results. These results of the total biofilm mass also inversely correlated to the data attained during the compliance results at T1 especially in those patients who showed no improvements in the oral hygiene skills subsequent to the establishment of the orthodontic treatment.

Table 1. Plaque Index (PI), Probing depth (PD), Bleeding on probing (BOP), Compliance and Total microbial biofilm mass, p values for changes between T1-T0; T2-T1 for each treatment group and controls.

	Time	Invisalign		Fixed Appliances		Control	
		Mean (SD)	P-value (Time x / Time 1)	Mean (SD)	P-value (Time x / Time 1)	Mean (SD)	P-value (Time x / Time 1)
PI	0	0,5 (0,51)	0,660	0,25 (0,44)	0,074	0,2 (0,41)	0,011
	1	0,35 (0,48)	1,000	0,95 (0,94)	1,000	0,45 (0,51)	1,000
	2	0,4 (0,59)	<0,001	1,15 (0,67)	0,060	0,45 (0,60)	0,024
PD (mm)	0	2 (0)	-	2,05 (0,22)	0,331	2,15 (0,36)	-
	1	2,15 (0,36)	1,000	2,5 (0,51)	1,000	2,15 (0,36)	-
	2	2,3 (0,47)	0,002	2,95 (0,60)	0,062	2,15 (0,36)	-
BOP	0	0,25 (0,44)	0,036	0,1 (0,3)	0,246	0,05 (0,22)	0,083
	1	0,4 (0,5)	1,000	0,6 (0,5)	1,000	0,25 (0,44)	1,000
	2	0,3 (0,47)	<0,001	0,85 (0,36)	0,139	0,2 (0,41)	<0,001
Compliance	0	-	-	-	-	-	-
	1	2,4 (0,82)	1,000	1,2 (0,41)	1,000	1,3 (0,47)	1,000
	2	2,3 (0,92)	<0,001	1,5 (0,68)	<0,001	1,4 (0,50)	<0,001
Biofilm	0	3,63 (0,80)	0,528	3,44 (1,03)	0,012	3,84 (0,27)	<0,001
	1	3,07 (0,83)	1,000	3,91 (0,87)	1,000	3,75 (0,57)	1,000
	2	2,94 (0,88)	0,003	3,71 (0,97)	0,272	3,62 (0,64)	<0,001

A p value of <0.05 was considered significant, bold p values are statistically significant.

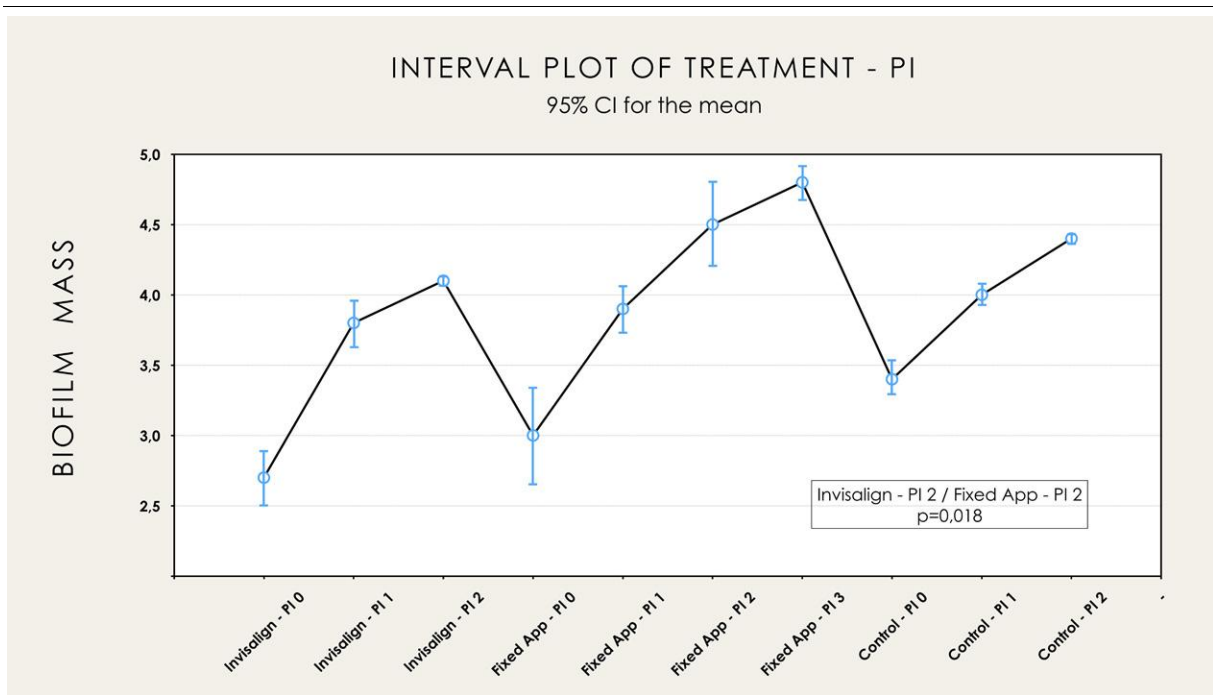


Figure 1. Flow chart illustrating the experimental procedure.

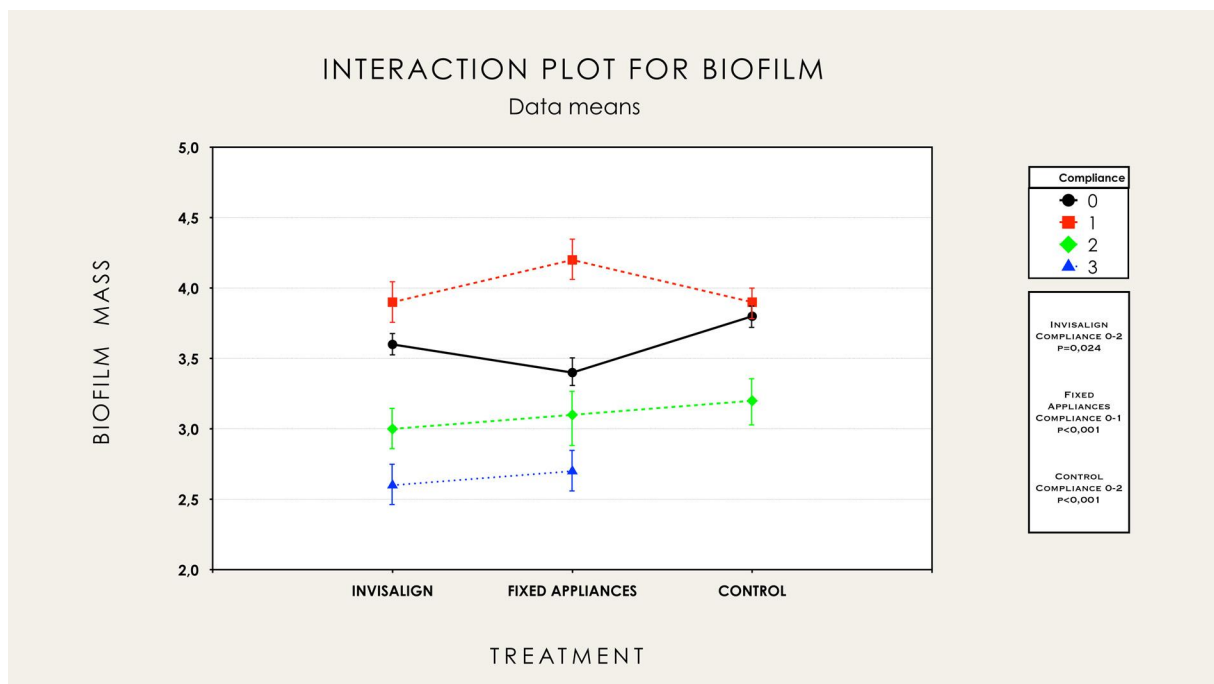


Figure 2. Molecular results progress divided by Treatment and PI.

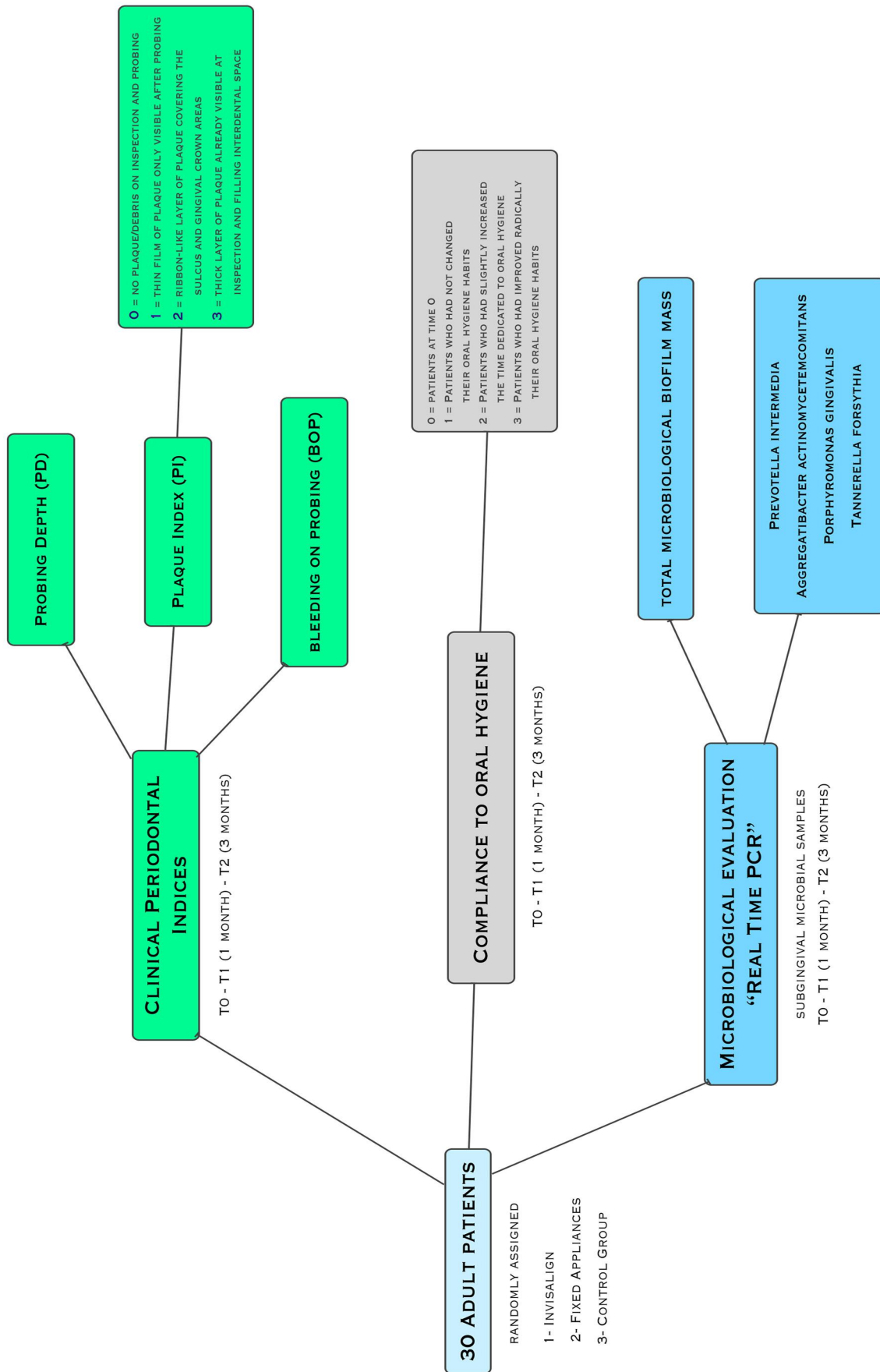


Figure 3. Interaction analysis between Treatment and Compliance.

DISCUSSION

This study was conducted over a period of three months following the study of months of treatment and a significant decrease of the same indices in the succeeding six months. Furthermore, since it has been demonstrated that the use of orthodontic bands and bonded tubes on molars may differently influence the accumulation of bacteria and the inflammatory response of periodontal tissues, bands were excluded and not employed in this study.^{5,26,27,28} The microbiological analysis of the total microbiological biofilm mass and the identification of periodontopathic species such as *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* performed in this study showed a low risk for periodontal disease in adult patients undergoing fixed or removable orthodontic therapy with Invisalign® System (Align Technology, Santa Clara, California). Therefore, the null hypothesis that the types of orthodontic appliances (fixed or removable) increase the risk for periodontal disease must be accepted. However, the total microbiological biofilm results were significantly higher in patients undergoing fixed orthodontic treatment and considerably correlated to the results obtained during the preliminary clinical assessment performed on the oral hygiene compliance of the patient, modified Plaque Index (PI), pocket probing depth (PD) and bleeding on probing (BOP). Hence, the second null hypothesis that the removable Invisalign® System offered no benefits on the bacteria plaque control (patients' oral hygiene compliance) and on the periodontal health status (periodontal indices) when compared to fixed orthodontic appliances must be rejected.

These results are in agreement with those reported by van Gastel et al.²⁹ who showed that bracket design may have a significant impact on the evolution of the surrounding microbial environment. Naranjo et al.¹⁷ conducted a study in

Ristic et al.² which found an increase of the clinical and microbiological indices using fixed appliances in the first three patients undergoing orthodontic treatment with fixed appliances over a period of three months showing that the presence of the brackets influenced the accumulation of plaque and the colonization of important periodontopathic bacteria such as *P. gingivalis*, *P. intermedia*, *Tannerella forsythensis* and *Fusobacterium species* which induced severe gingival inflammation and high bleeding scores. Van Gastel et al.³⁰ found that placement of fixed orthodontic appliances had an influence both on microbial and clinical periodontal parameters, which were only partly normalized, 3 months following the removal of the appliances. Lo Bue et al.³¹ suggested a pathogenetic role for anaerobic bacteria as responsible of gingivitis and periodontal damage during orthodontic therapies, and that monitoring anaerobic bacteria is highly recommended following the placement of orthodontic appliances. In this study, only one sample treated with fixed orthodontic appliances was found positive to *Aggregatibacter actinomycetemcomitans* at T1 and T2 while, all the other subjects whose bacterial samples were analyzed with Real Time PCR were found negative for the periodontopathic anaerobes. These differences may be correlated to subjects involved in this study who showed a high level of compliance in oral hygiene which positively influenced the periodontal health during the orthodontic treatment. Petti et al.¹⁰ compared the effect of fixed and removable orthodontic treatments on supra and subgingival microflora in adolescents (7-15 years old). The authors showed that during the first 6 months of treatment no gingivitis and periodontitis occurred in patients who were well motivated and showed a high compliance to oral hygiene. However, in this study it was possible to attain a microbial biofilm mass up to < 100 bacteria/PCR in subjects undergoing treatment with the Invisalign® with a

strong influence of compliance to oral hygiene on both of the microbial biofilm mass ($p < 0.001$) and PI ($p = 0.001$). Patients undergoing treatment with aligners had to remove them many times during the day, for eating or simply drinking beverages containing sugar. This habit turn them more careful on their oral hygiene procedures before wearing back the aligners and explains their higher compliance during the treatment, also compared to the control group.

It is important to remark that patients had to wear removable aligners for 20 hours a day and could therefore perform domiciliary oral hygiene procedures without obstacles. This work represents a preliminary study due to the sample size, nevertheless it showed different interesting outcomes, particularly that the different types of orthodontic treatment could have a direct influence on microbial biofilm level mainly in those subjects who had a poor oral hygiene compliance. The analysis of the interaction between oral hygiene compliance and orthodontic treatments (Figure 2) showed that the increasing of total biofilm mass in patients who showed no improvements in the oral hygiene skills (Compliance 1) was much higher in the group treated with fixed orthodontic appliances compared to the one with removable aligners. In other words, patients with a low level of oral hygiene could maintain better periodontal health whilst undergoing an orthodontic treatment with removable aligners rather than one with fixed appliances. On the contrary, the higher the compliance level the lower the microbial biofilm mass, PB and BOP in both types of orthodontic treatments. Conversely, this study has demonstrated that patients treated with fixed orthodontic appliances had a higher value of both PD and BOP at T2 in comparison to those obtained at T1 (1st month assessment) and a significant statistical correlation to the increase of microbial biofilm mass ($p < 0.001$).

CONCLUSIONS

The results of this preliminary study confirmed that oral hygiene compliance plays an important role in maintaining a good periodontal health independent of the type of orthodontic treatment performed (fixed or removable). Patients treated with the Invisalign® System may maintain a lower level of microbial biofilm mass, even with poor oral hygiene compliance.

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