Journal of Orofacial Orthopedics/ Fortschritte der Kieferorthopädie

Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances.

--Manuscript Draft--

Manuscript Number:	JOOF-D-14-00034R2
Full Title:	Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances.
Article Type:	Original Article
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Abstract:	Objectives: To explore the microbiological and periodontal changes occurring in adolescents during 12 months of orthodontic therapy with removable aligners (Invisalign®) and with fixed appliances. Subjects and Methods: During the years 2012-2013, 50 teenagers aged 10-18 years with similar orthodontic starting conditions participated in this trial in a university clinic of northern Italy. After receiving professional oral hygiene and instructions on a standardized oral hygiene protocol, the adolescents were randomly assigned to either orthodontic treatment with traditional fixed brackets (n=25) or to treatment with Invisalign® aligners (n=25). Subgingival microbiological samples, probing depth (PD), plaque index (PI), and bleeding on probing (BOP) were recorded from the mesio-vestibular subgingival sulcus of the upper right first molar and left central incisor at the beginning of treatment and 3, 6, and 12 months later. Compliance to oral hygiene procedures, full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were assessed at the beginning of treatment and 12 months later. Two sample independent t-tests and Chi-square test were used to study whether the indices of periodontal health differed for the teenagers by experimental conditions. Results: None of the patients resulted positive for the periodontal anaerobes analyzed. PI, PD, BOP, FMPS and FMBS resulted significantly lower and compliance to oral hygiene resulted significantly higher in the group treated with Invisalign® compared to the group treated with fixed brackets. Conclusion: Teenagers treated with removable appliances show better compliance to oral hygiene, less plaque, and gingival inflammatory reactions than peers with fixed appliances.

Response to Reviewers:	Thank you for considering for publication our contribution “Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances.” The comments of the reviewers are fair and useful to enhance the quality of our manuscript. We sincerely hope that the reviewers will find our observations enough explained. The changes to the original manuscript are underlined in order to facilitate the discussion with the reviewers. Sincerely, Gian Marco Abbate, corresponding author. Reviewer #1: Sealed envelopes were used to assign each teenager to the treatment group in order to blind the decision on the treatment to perform. The local ethical committee was consulted and expressed their authorization n.826 03/10/2011. More details on the microbiological procedure were included in materials and methods: Each paper point was suspended in 50 ul of pure dimethyl sulfoxide (DMSO) and centrifuged for 30 seconds. 2 µl of this was used as DNA suspension for real time PCR reactions. 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 cyclesof0secat95°C,10secat50°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 Tm peaks. The amount of bacterial DNA in the samples was calculated following sequent formula [C = q*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. A statement for potential conflicts of interest was included at the end of the manuscript. Reviewer #2: In order to avoid to give the impression to the readers that aligners are "better" than fixed appliances, in the discussion the following concept was underlined: This study did not aim to evaluate the orthodontic outcome of two different approaches (fixed and removable) but the periodontal health in a population of teenagers in which both techniques could be performed. This can represent for the clinician a possibility to consider different treatment options, mainly in teenagers in which compliance to oral hygiene can be difficult to obtain and esthetic concern is particularly present. The absence of drop outs for poor wearing time was considered in the discussion with the following concept: No drop outs for poor wearing time of the aligners were observed and this can be explained with the strong motivation of the patients. Teenagers treated with removable aligners need to be continually motivated mainly after the first months of treatment in order to avoid failure due to insufficient wearing time.

Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances.

Keywords: periodontal health, teenagers, Invisalign®, fixed appliances, plaque score, bleeding score, probing depth, compliance, oral hygiene.

Abstract

Objectives: To explore the microbiological and periodontal changes occurring in adolescents during 12 months of orthodontic therapy with removable aligners (Invisalign®) and with fixed appliances.
Subjects and Methods: During the years 2012-2013, 50 teenagers aged 10–18 years with similar orthodontic starting conditions participated in this trial in a university clinic of northern Italy. After receiving professional oral hygiene and instructions on a standardized oral hygiene protocol, the adolescents were randomly assigned to either orthodontic treatment with traditional fixed brackets (n=25) or to treatment with Invisalign® aligners (n=25). Subgingival microbiological samples, probing depth (PD), plaque index (PI), and bleeding on probing (BOP) were recorded from the mesio-vestibular subgingival sulcus of the upper right first molar and left central incisor at the beginning of treatment and 3, 6, and 12 months later. Compliance to oral hygiene procedures, full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were assessed at the beginning of treatment and 12 months later. Two sample independent t-tests and Chi-square test were used to study whether the indices of periodontal health differed for the teenagers by experimental conditions.
Results: None of the patients resulted positive for the periodontal anaerobes analyzed. PI, PD, BOP, FMPS and FMBS resulted significantly lower and compliance to oral hygiene resulted significantly higher in the group treated with Invisalign® compared to the group treated with fixed brackets.
Conclusion: Teenagers treated with removable appliances show better compliance to oral hygiene, less plaque, and gingival inflammatory reactions than peers with fixed appliances.

Introduction

Treatment with fixed orthodontic appliances increases the risk of white spot lesions, caries, and gingivitis during and after the orthodontic therapy. In particular, inflammatory reaction of gingival tissue has often been reported, with an increase in plaque index, bleeding and probing depth, in correspondence of teeth with orthodontic brackets [3,4,30]. It seems that the main factor for an increased accumulation of dental plaque and inflammatory response is the presence of retentive places around the components of fixed appliances attached to the teeth [9,22]. Moreover, a significant loss of periodontal attachment has been reported after fixed orthodontic treatment [11]. Consequently, patients with fixed appliances should follow a very rigid oral hygiene protocol to avoid these side effects.
Parallel to the clinical investigations of periodontal indices, microbiological studies of the composition of the subgingival dental plaque in patients with fixed orthodontic brackets revealed significant changes, suggesting that the treatment may stimulate the growth of a subgingival flora where some periodontopathogenic bacterial strains are prevalent [20], such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides*

forsythus, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum and Treponema denticola [25].

Unlike fixed dental appliances, removable orthodontic appliances can be worn off and allow patients to practice oral hygiene procedures in ideal conditions [26,28]. Invisalign® (Align Technology, Santa Clara, Calif.) is a method for resolution of orthodontic problems that exploits the action of a series of transparent removable aligners made by polymer material to gradually move the teeth in the position previously planned in a "virtual treatment", using a proprietary software program. These aligners cover all the teeth plus the marginal aspect of the gingiva and should be worn ideally 20-22 hours per day and removed only for eating, drinking, and tooth brushing or flossing [6]. Invisalign®, being removable, has the potential of not hindering oral hygiene, since patients do not have the obstructions caused by brackets, bands or archwires. Miethke and colleagues showed that using Invisalign® lead to an improvement of clinical periodontal status in adult patients compared to fixed lingual orthodontic devices, because of the absence of the biofilm typically detected in patients wearing fixed appliances [17]. The Invisalign® treatment seems to offer several advantages in terms of maintenance of oral hygiene [15]. No signs of gingivitis and improvement of gingival and plaque indices have been reported in different studies with patients undergoing orthodontic therapy with removable aligners [16,17,23].

Orthodontic therapy with fixed appliances has certain inherent drawbacks, including dental and soft tissue discomfort, and poor esthetics. Teenage patients may be particularly reluctant to undergo fixed-appliance treatment due to these drawbacks and for other social reasons [12,27]. Few studies in the literature take into account the effects of orthodontic therapy on periodontal indices and subgingival flora in teenagers, in which compliance to oral hygiene often represents a great challenge for the clinician.

The aim of this clinical trial was to explore the microbiological and periodontal changes occurring in adolescent patients during the first 12 months of orthodontic therapy with fixed appliances and with the Invisalign® system.

Materials and methods

This study included 50 teenagers (10-18 aged) of both sexes referred in 2012-2013 to the post-graduate clinic of the Department of Orthodontics, School of Dentistry, University of Insubria, Varese, Italy. The subjects fulfilled the following criteria for participation: non smokers, good general and periodontal health, absence of extensive dental restorations or adhesive fixed partial dentures, lack of antibiotic therapy 3 months before the beginning of the study and during the study, and no use of anti-plaque and oral anti-septic solutions during the trial. The orthodontic treatment plan did not include tooth extractions or other mechanics needing the use of bands on molars. For each patient a parent (or curator) signed a written consent to the therapy and the clinical trial.

One month before the orthodontic therapy, professional oral hygiene was performed on all the patients who also received instructions on a standardized oral hygiene protocol. Using sealed envelopes the teenagers were randomly assigned to the two experimental groups: 25 patients were treated with Invisalign® aligners (group 1) and 25 with fixed orthodontic appliances (group 2). The fixed orthodontic treatment was performed with directly bonded brackets on incisors, canines and premolars, and

orthodontic tubes directly bonded on the first molars. Invisalign® aligners were delivered to patients with instructions to be worn 20-22 hours per day and removed only for eating, drinking and tooth brushing. The patients had to wear each pair of aligners for two weeks before moving on to the next pair in the series.

The health status of the periodontal tissues was determined by using periodontal indices and subgingival microbiological analysis at the beginning of the orthodontic therapy (T0), 3 months (T3), 6 months (T6) and 12 months (T12) after the start of the treatment. All the clinical measurements were performed by the same operator at each visit, strictly respecting international criteria for the determination of periodontal indices scores [24]. Full mouth plaque score (FMPS), as percentage of dental surfaces with detectable plaque, and full mouth bleeding score (FMBS), as percentage of sites with bleeding on probing, were recorded in six sites (mesio-vestibular, vestibular, disto-vestibular, mesio-lingual, lingual, disto-lingual) of all teeth, at T0 and T12.

The subgingival microbiological samples, probing depth (PD), plaque index (PI) [14] and bleeding on probing (BOP) were recorded from the mesio-vestibular subgingival sulcus of the upper right first molar and the upper left central incisor [18], at T0, T3, T6 and T12. 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). BOP was registered 20 seconds after probing (absent=0, present=1) [29]. PI was assessed according to the plaque accumulation in the gingival area in four grades [14]:

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 sulcus and gingival crown areas but not filling interdental space
3 = thick layer of plaque already visible at inspection and filling interdental space

Oral hygiene compliance was assessed in each treatment group at the beginning of the study (T0) and at the end of the treatment (T12) interviewing the patients on the time spent for home oral care procedures using the following scoring criteria [13]:

(0) patients showing no improvement in oral hygiene compliance
(1) patients showing a slightly increased oral hygiene compliance
(2) patients showing a significant improvement in oral hygiene compliance

In order to standardize the procedure the evaluation was performed by the same operator who taught the initial procedures of oral hygiene to the teenagers and who collected the periodontal indices.

The microbiological samples were collected right after recording PI but prior to the other clinical procedures. Following microbiological sampling, probing depth was measured and bleeding on probing registered. The microbiological status was determined by investigating presence or absence of four periodontopathic anaerobes (*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 gingival sulcus for 30 seconds. After insertion, paper points were closed into a test tube, refrigerated at -20° C and sent to the O.B.L (Oral Biotechnology Laboratory) University of Cagliari, Italy, where the microbiological analysis was performed. Periodontal pathogens were detected by real time PCR procedures [5,21]. The molecular protocols used in this study have been previously described [7,19].

Descriptive statistical analyses were performed to summarize the main characteristics of the patients in the two experimental conditions under comparison. Two sample independent t-tests were used to study whether the means of full mouth plaque and bleeding scores differed for the subjects by experimental conditions, at the beginning and at the end of the study. Two sample independent t-tests were used to study whether the means of plaque index, probing depth, and bleeding on probing differed for the subjects by experimental conditions, at each visit and at different sites (molar and incisor). The differences of bleeding on probing among the teenagers of group 1 and 2 were determined using Chi-square test. The level of significance was set at $P < 0.05$. All analyses were performed using the statistical package Stata version 12.

Results

The experimental procedure was completed without complications and was well tolerated by the patients. Three dropouts were recorded in the group treated with Invisalign® (group 1) due to the use of antibiotics for systemic diseases, that caused also impossibility to perform all the clinical and microbiological procedures. As expected in a randomized study, at the beginning of treatment most of the indices under study were similar in the two experimental groups. Only indexes of FMPS and of BOP for the incisors were different in the two groups at baseline, with higher values in patients treated with the Invisalign® system.

From the beginning to the end of orthodontic treatment, full mouth plaque score has tripled and full mouth bleeding score has doubled in adolescents with brackets (group 2) while both have slightly decreased in adolescents wearing Invisalign® aligners (Table 1). Also the plaque and bleeding indices recorded on incisors and molars increased during fixed treatment, while a reduction was observed during treatment with the Invisalign® system (Table 2 and 3). Probing depth increased in all the treated patients, particularly in the group 2 and slightly less in group 1 (Table 2).

At the end of the orthodontic therapy FMPS and FMBS resulted significantly lower ($p<0.01$) in group 1 in comparison to group 2 (Tab. 1). PI, PD and BOP at the mesio-vestibular subgingival sulcus of the upper right first molar and the upper left central incisor resulted significantly lower ($p<0.01$) in group 1 than in group 2 at T3, T6 and T12 (Tab 2, 3). None of the patients resulted positive for the four anaerobes (*Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia*) analyzed during the 12 months of therapy. Compliance to oral hygiene evaluated at T12 as score 0-2 (Tab. 4) resulted significantly higher in group 1 (mean value 1.9) when compared to group 2 (mean value 1.2). About half of adolescents have maintained the same level of oral hygiene after 12 months of orthodontic treatment. About one-third of the sample (n=17) has slightly improved oral hygiene procedures, while only 8% (n=4) has greatly improved. The increase in oral hygiene procedures resulted statistically associated to the type of treatment: $p<0,01$ (Tab. 4). In fact, among patients with fixed brackets, only one out of five had slightly improved its oral hygiene habits (n=5) while no adolescent had greatly improved. On the contrary, among patients with Invisalign®, 70% (n=12) had slightly improved and about 20% (n=4) had greatly improved.

Discussion

In this experimental study, adolescents who wore Invisalign® aligners (group 1) showed better indices of periodontal health than their peers with fixed attachments (group 2), after an equal period of orthodontic treatment.

These findings seem to disagree with Alstad and Zachrisson [1] that compared the periodontal condition of adolescents with fixed appliances with untreated patients and reported no statistically significant difference in the mean plaque score and the gingival condition. In that study professional tooth cleaning was done at each visit, actually reducing the effect of the patient's compliance to oral hygiene. In the present study each patient received professional oral care and standardized hygiene instructions one month before the beginning of the therapy, then for the 12 months of orthodontic therapy no further interventions were performed. On equal compliance the results of the periodontal indices in group 1 were significantly better, confirming that oral hygiene procedures without the impediments of the fixed appliances are facilitated in the adolescent patient, in accordance with Miethke and Vogt's observations in adults [16].

Longitudinal studies have shown that adverse changes in the subgingival microflora occur shortly after placement of appliances and these are usually accompanied by plaque, bleeding, and probing depths [8,10]. Increase of plaque accumulation, BOP and probing depth could be observed at the incisor and molar site in group 2 (tab 2 and 3), and this increase appeared to be continuous from the beginning of the therapy and reached the maximum values at the end of the study even if no patient showed attachment loss or periodontitis. Ristic and coworkers [22] reported an increase of plaque index, gingival index, gingival bleeding index and pocket probing depth on incisors and molars to a maximum of 3 months after fixed-appliance treatment and a tendency of decrease of measured clinical indices 6 months after the beginning of orthodontic therapy. In the study of Ristic and coworkers no professional hygiene was performed before application of fixed appliances in order to prevent the possibility of eradicating periodontal pathogen microorganisms from the subgingival environment and periodontopathic anaerobes like *P. intermedia, A. actinomycetemcomitans and P. gingivalis* could be detected already at the beginning of the orthodontic treatment [22]. None of the patients of the present study resulted positive for one of the four periodontopathic anaerobes analysed, even after 12 months of treatment. A completely opposite trend could be observed for the patients randomized to group 1 in which PI and BOP decreased from T0 to T12, while probing depth resulted stable (Tab 2 and 3). Miethke and coworkers [16] reported a decrease of PI in adults treated with the Invisalign® system but could still not find out differences in periodontal health between treatment with fixed or removable appliances. In that study the patients received repeated instructions, motivation and professional oral hygiene support that could explain the lack of difference between the two treatment options.

Compliance to oral hygiene evaluated interviewing the patients on the time spent for home oral care procedures was significantly greater in the patients of group 1(Tab. 4). Patients undergoing treatment with the removable aligners had to remove them before eating and use to perform oral hygiene procedures before wearing them back. This can explain the higher score of compliance recorded in this group in which patients reported a significant change in their oral hygiene habits when compared to group 2. This way of evaluating the compliance has been performed before in a study on the Invisalign® technique in adults with similar results [13]. Other studies exist where the microbiological and periodontal responses in adolescents and adults have been compared and generally adults resulted with better supragingival plaque control than adolescents [2]. It has been suggested that adults have longer clinical crowns and the

attachments are further away from the gingival tissues and adolescents often have increased hormonal levels associated with puberty which may favor gingival inflammation. The increased cooperation in terms of brushing generated therefore an improvement of the overall periodontal health. An excellent oral health in Invisalign® patients has been reported also by Schaefer and Braumann [23] and was according to these authors mainly due to an improvement in the patient's awareness of oral hygiene, without the need for regular oral hygiene instructions. This aspect can be particularly important in teen patients often difficult to motivate in keeping a good level of oral home care.

An important limitation of this study is the small sample size. However, the similarity of the indexes of periodontal health at baseline between the two experimental groups prompts their comparability and therefore the validity of findings. The only two indices distributed differently in the two experimental groups indicate a worse baseline periodontal situation in group 1 compared to group 2, in this situation the advantage of treatment with Invisalign® compared to treatment with fixed appliances for periodontal health would rather be underestimated.

Another limitation of the study is that microbiological analysis could be performed only in two sites (molar and incisor) and only four bacteria could be analyzed. This was due to the need to contain costs. However, more extensive microbiological procedures performed in all the sites of the oral cavity could be advisable. Another limitation is the duration of the follow-up. Longitudinal data at the end of treatment and long-term evolution of periodontal health are not considered in this preliminary study. This could be an interesting field of research for future trials.

Strength can be found in the analysis of the compliance to oral hygiene which actually needs to be classified and scored, representing a precondition to preserve periodontal health during the orthodontic therapy.

The results of this study suggest that teenagers treated with removable appliances show better compliance to oral hygiene, less plaque, and gingival inflammatory reactions than peers with fixed appliances, during 12 months of orthodontic therapy.

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Legends

Tab 1. Comparison of full mouth plaque and bleeding scores for patients treated with Invisalign® and fixed orthodontic appliances at the beginning of treatment and 12 months after.

Tab. 2 Comparison of plaque index and probing depth for patients treated with Invisalign® and fixed orthodontic appliances at the beginning of treatment, 3, 6, and 12 months after.

Tab. 3 Comparison of bleeding on probing for patients treated with Invisalign® and fixed orthodontic appliances at the beginning of treatment, 3, 6, and 12 months after.

Tab. 4 Comparison of compliance to oral hygiene for patients treated with Invisalign® and fixed orthodontic appliances 12 months after the beginning of treatment (n).

Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances: a preliminary study.

Abstract

Objectives: To explore the microbiological and periodontal changes occurring in adolescents during 12 months of orthodontic therapy with removable aligners and with fixed appliances.
Material and Methods: During the years 2012-2013, 50 teenagers aged 10–18 years with similar orthodontic starting conditions participated in this trial in a university clinic of northern Italy. After receiving professional oral hygiene and instructions on a standardized oral hygiene protocol, the adolescents were randomly assigned to either orthodontic treatment with traditional fixed brackets (n=25) or to treatment with Invisalign® aligners (n=25). Subgingival microbiological samples, probing depth (PD), plaque index (PI), and bleeding on probing (BOP) were recorded from the mesio-vestibular subgingival sulcus of the upper right first molar and left central incisor at the beginning of treatment and 3, 6, and 12 months later. Compliance to oral hygiene procedures, full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were assessed at the beginning of treatment and 12 months later. Two sample independent t-tests and Chi-square test were used to study whether the indices of periodontal health differed for the teenagers by experimental conditions.
Results: None of the patients resulted positive for the periodontal anaerobes analyzed. PI, PD, BOP, FMPS and FMBS resulted significantly lower and compliance to oral hygiene resulted significantly higher in the group treated with Invisalign® compared to the group treated with fixed brackets.
Conclusions: Teenagers treated with removable appliances show better compliance to oral hygiene, less plaque, and gingival inflammatory reactions than peers with fixed appliances.

Introduction

Treatment with fixed orthodontic appliances increases the risk of white spot lesions, caries, and gingivitis during and after the orthodontic therapy. In particular, inflammatory reaction of gingival tissue has often been reported, with an increase in plaque index, bleeding and probing depth, in correspondence of teeth with orthodontic brackets (Zachrisson et al. 1974; Bollen et al., 2008). It seems that the main factor for an increased accumulation of dental plaque and inflammatory response is the presence of retentive places around the components of fixed appliances attached to the teeth (Ristic et al. 2007; Gomes et al, 2007). Moreover, a significant loss of periodontal attachment has been reported after fixed orthodontic treatment (Janson G et al. 2003). Consequently, patients with fixed appliances should follow a very rigid oral hygiene protocol to avoid these side effects.
Parallel to the clinical investigations of periodontal indices, microbiological studies of the composition of the subgingival dental plaque in patients with fixed orthodontic brackets revealed significant changes, suggesting that the treatment may stimulate the growth of a subgingival flora where some periodontopathogenic bacterial strains are prevalent (Petti et al. 1997), such as Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum and Treponema denticola (Socransky et al., 1992).

Unlike fixed dental appliances, removable orthodontic appliances can be worn off and allow patients to practice oral hygiene procedures in ideal conditions (Taylor et al., 2003; Turatti et al., 2006). Invisalign® (Align Technology, Santa Clara, Calif.) is a method for resolution of orthodontic problems that exploits the action of a series of transparent removable aligners made by polymer material to gradually move the teeth in the position previously planned in a "virtual treatment", using a proprietary software program. These aligners cover all the teeth plus the marginal aspect of the gingiva and should be worn ideally 20-22 hours per day and removed only for eating, drinking, and tooth brushing or flossing (Boyd RL et al. 2000). Invisalign, being removable, has the potential of not hindering oral hygiene, since patients do not have the obstructions caused by brackets, bands or archwires. Miethke and colleagues showed that using Invisalign lead to an improvement of clinical periodontal status in adult patients compared to fixed lingual orthodontic devices, because of the absence of the biofilm typically detected in patients wearing fixed appliances (Miethke et al. 2007). The Invisalign treatment seems to offer several advantages in terms of maintenance of oral hygiene (Mampieri and Giancotti, 2013). No signs of gingivitis and improvement of gingival and plaque indices have been reported in different studies with patients undergoing orthodontic therapy with removable aligners (Schaefer et al., 2010; Miethke et al., 2005, 2007).

Orthodontic therapy with fixed appliances has certain inherent drawbacks, including dental and soft tissue discomfort, and poor esthetics. Teenage patients may be particularly reluctant to undergo fixed-appliance treatment due to these drawbacks and for other social reasons (Tuncay et al., 2013). Few studies in the literature take into account the effects of orthodontic therapy on periodontal indices and subgingival flora in teenagers, in which compliance to oral hygiene often represents a great challenge for the clinician.

The aim of this preliminary clinical trial was to explore the microbiological and periodontal changes occurring in adolescent patients during the first 12 months of orthodontic therapy with fixed appliances and with the Invisalign® system.

Materials and methods

This study included 50 teenagers (10-18 aged) of both sexes referred in 2012-2013 to the post-graduate clinic of the Department of Orthodontics, School of Dentistry, University of Insubria, Varese, Italy. The subjects fulfilled the following criteria for participation: non smokers, good general and periodontal health, absence of extensive dental restorations or adhesive fixed partial dentures, lack of antibiotic therapy 3 months before the beginning of the study and during the study, and no use of anti-plaque and oral anti-septic solutions during the trial. The orthodontic treatment plan did not include tooth extractions or other mechanics needing the use of bands on molars. For each patient a parent (or curator) signed a written consent to the therapy and the clinical trial. An authorization of the local ethical committee was obtained.

One month before the orthodontic therapy, professional oral hygiene was performed on all the patients who also received instructions on a standardized oral hygiene protocol. Using sealed envelopes, in order to blind the decision on the treatment to perform, the teenagers were randomly assigned to the two experimental groups: 25 patients were treated with Invisalign® aligners (group 1) and 25 with fixed orthodontic appliances (group 2). The fixed orthodontic treatment was performed with directly bonded brackets on incisors, canines and premolars, and orthodontic tubes directly bonded on the first molars. Invisalign® aligners were delivered to patients

with instructions to be worn 20-22 hours per day and removed only for eating, drinking and tooth brushing. The patients had to wear each pair of aligners for two weeks before moving on to the next pair in the series.

The health status of the periodontal tissues was determined by using periodontal indices and subgingival microbiological analysis at the beginning of the orthodontic therapy (T0), 3 months (T3), 6 months (T6) and 12 months (T12) after the start of the treatment. All the clinical measurements were performed by the same operator at each visit, strictly respecting international criteria for the determination of periodontal indices scores (Sillness J, Loe H 1964). Full mouth plaque score (FMPS), as percentage of dental surfaces with detectable plaque, and full mouth bleeding score (FMBS), as percentage of sites with bleeding on probing, were recorded in six sites (mesio-vestibular, vestibular, disto-vestibular, mesio-lingual, lingual, disto-lingual) of all teeth, at T0 and T12.

The subgingival microbiological samples, probing depth (PD), plaque index (PI) (Löe H, Silness J 1963) and bleeding on probing (BOP) were recorded from the mesio-vestibular subgingival sulcus of the upper right first molar and the upper left central incisor (Mumghamba EGS et al. 2004), at T0, T3, T6 and T12. 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). BOP was registered 20 seconds after probing (absent=0, present=1) (van Gastel J et al. 2008). PI was assessed according to the plaque accumulation in the gingival area in four grades:

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 sulcus and gingival crown areas but not filling interdental space
3 = thick layer of plaque already visible at inspection and filling interdental space

Oral hygiene compliance was assessed in each treatment group at the beginning of the study (T0) and at the end of the treatment (T12) interviewing the patients on the time spent for home oral care procedures using the following scoring criteria:

(0) patients showing no improvement in oral hygiene compliance
(1) patients showing a slightly increased oral hygiene compliance
(2) patients showing a significant improvement in oral hygiene compliance

In order to standardize the procedure the evaluation was performed by the same operator who taught the initial procedures of oral hygiene to the teenagers and who collected the periodontal indices.

The microbiological samples were collected right after recording PI but prior to the other clinical procedures. Following microbiological sampling, probing depth was measured and bleeding on probing registered. The microbiological status was determined by investigating presence or absence of four periodontopathic anaerobes (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 gingival sulcus for 30 seconds. After insertion, paper points were closed into a test tube, refrigerated at -20° C and sent to the Department of Microbiology (DNA Sequencing Service), University of Cagliari, Italy, where the microbiological analysis was performed. Periodontal pathogens were detected by real time PCR procedures (Bommarito S et al. 2000;

Rasmussen R 2001). The molecular protocols used in this study have been previously described (Orrù G et al. 2006; Denotti G et al. 2009). Each paper point was suspended in 50 ul of pure dimethyl sulfoxide (DMSO) and centrifuged for 30 seconds. 2 µl of this was used as DNA suspension for real time PCR reactions. 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 Tm peaks. The amount of bacterial DNA in the samples was calculated following sequent formula [C = q*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.
Descriptive statistical analyses were performed to summarize the main characteristics of the patients in the two experimental conditions under comparison. Two sample independent t-tests were used to study whether the means of full mouth plaque and bleeding scores differed for the subjects by experimental conditions, at the beginning and at the end of the study. Two sample independent t-tests were used to study whether the means of plaque index, probing depth, and bleeding on probing differed for the subjects by experimental conditions, at each visit and at different sites (molar and incisor). The differences of bleeding on probing among the teenagers of group 1 and 2 were determined using Chi-square test. The level of significance was set at $P < 0.05$. All analyses were performed using the statistical package Stata version 12.

Results

The experimental procedure was completed without complications and was well tolerated by the patients. Three dropouts were recorded in the group treated with Invisalign® due to the use of antibiotics for systemic diseases, that caused also impossibility to perform all the clinical and microbiological procedures. As expected in a randomized study, at the beginning of treatment most of the indices under study were similar in the two experimental groups. Only indexes of FMPS and of BOP for the incisors were different in the two groups at baseline, with higher values in patients treated with Invisalign®.
From the beginning to the end of orthodontic treatment, full mouth plaque score has tripled and full mouth bleeding score has doubled in adolescents with brackets while both have slightly decreased in adolescents wearing Invisalign® aligners (Table 1). Also the plaque and bleeding indices recorded on incisors and molars increased during fixed treatment, while a reduction was observed during treatment with the Invisalign® system (Table 2 and 3). Probing depth increased in all the treated patients,

particularly in the group with fixed attachments and slightly less among the Invisalign® patients (Table 2).
At the end of the orthodontic therapy FMPS and FMBS resulted significantly lower ($p<0.01$) in group 1 in comparison to group 2 (Tab. 1). PI, PD and BOP at the mesio-vestibular subgingival sulcus of the upper right first molar and the upper left central incisor resulted significantly lower ($p<0.01$) in group 1 than in group 2 at T3, T6 and T12 (Tab 2, 3). None of the patients resulted positive for the four anaerobes (Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia) analyzed during the 12 months of therapy. Compliance to oral hygiene evaluated at T12 as score 0-2 (Tab. 4) resulted significantly higher in group 1 (mean value 1.9) when compared to group 2 (mean value 1.2). About half of adolescents have maintained the same level of oral hygiene after 12 months of orthodontic treatment. About one-third of the sample (n=17) has slightly improved oral hygiene procedures, while only 8% (n=4) has greatly improved. The increase in oral hygiene procedures resulted statistically associated to the type of treatment: $p<0,01$ (Tab. 4). In fact, among patients with fixed brackets, only one out of five had slightly improved its oral hygiene habits (n=5) while no adolescent had greatly improved. On the contrary, among patients with Invisalign®, 70% (n=12) had slightly improved and about 20% (n=4) had greatly improved.

Discussion

In this experimental study, adolescents who wore Invisalign® aligners showed better indices of periodontal health than their peers with fixed attachments, after an equal period of orthodontic treatment.
These findings seem to disagree with Alstad and Zachrisson that compared the periodontal condition of adolescents with fixed appliances with untreated patients and reported no statistically significant difference in the mean plaque score and the gingival condition. In that study professional tooth cleaning was done at each visit, actually reducing the effect of the patient's compliance to oral hygiene (Alstad et al., 1979). In the present study each patient received professional oral care and standardized hygiene instructions one month before the beginning of the therapy, then for the 12 months of orthodontic therapy no further interventions were performed. On equal compliance the results of the periodontal indices in group 1 were significantly better, confirming that oral hygiene procedures without the impediments of the fixed appliances are facilitated in the adolescent patient, in accordance with Miethke and Vogt's observations in adults (Miethke at al., 2007).
Longitudinal studies have shown that adverse changes in the subgingival microflora occur shortly after placement of appliances and these are usually accompanied by plaque, bleeding, and probing depths (Diamanti-Kipioti et al., 1987; Huser et al., 1990). Increase of plaque accumulation, BOP and probing depth could be observed at the incisor and molar site in group 2 (tab 2 and 3), and this increase appeared to be continuous from the beginning of the therapy and reached the maximum values at the end of the study even if no patient showed attachment loss or periodontitis. Ristic and coworkers reported an increase of plaque index, gingival index, gingival bleeding index and pocket probing depth on incisors and molars to a maximum of 3 months after fixed-appliance treatment and a tendency of decrease of measured clinical indices 6 months after the beginning of orthodontic therapy (Ristic et al., 2007). In the study of Ristic and coworkers no professional hygiene was performed before

application of fixed appliances in order to prevent the possibility of eradicating periodontal pathogen microorganisms from the subgingival environment and periodontopathic anaerobes like P. intermedia, A. actinomycetemcomitans and P. gingivalis could be detected already at the beginning of the orthodontic treatment. None of the patients of the present study resulted positive for one of the four periodontopathic anaerobes analysed, even after 12 months of treatment. A completely opposite trend could be observed for the patients randomized to group 1 in which PI and BOP decreased from T0 to T12, while probing depth resulted stable (Tab 2 and 3). Miethke and coworkers reported a decrease of PI in adults treated with the Invisalign® system but could still not find out differences in periodontal health between treatment with fixed or removable appliances (Miethke et al., 2005). In that study the patients received repeated instructions, motivation and professional oral hygiene support that could explain the lack of difference between the two treatment options.

Compliance to oral hygiene evaluated interviewing the patients on the time spent for home oral care procedures was significantly greater in patients treated with the Invisalign® system (Tab. 4). Patients undergoing treatment with the removable aligners had to remove them before eating and use to perform oral hygiene procedures before wearing them back. This can explain the higher score of compliance recorded in group 1 in which patients reported a significant change in their oral hygiene habits when compared to group 2. This way of evaluating the compliance has been performed before in a study on the Invisalign® technique in adults with similar results (Levrini et al. 2013). Other studies exist where the microbiological and periodontal responses in adolescents and adults have been compared and generally adults resulted with better supragingival plaque control than adolescents (Atack et al., 1996). It has been suggested that adults have longer clinical crowns and the attachments are further away from the gingival tissues and adolescents often have increased hormonal levels associated with puberty which may favor gingival inflammation. The increased cooperation in terms of brushing generated therefore an improvement of the overall periodontal health. An excellent oral health in Invisalign® patients has been reported also by Schaefer and Braumann and was according to these authors mainly due to an improvement in the patient's awareness of oral hygiene, without the need for regular oral hygiene instructions (Schaefer, Braumann, 2010). This aspect can be particularly important in teen patients often difficult to motivate in keeping a good level of oral home care.

No drop outs for poor wearing time of the aligners were observed and this can be explained with the strong motivation of the patients. Teenagers treated with removable aligners need to be continually motivated mainly after the first months of treatment in order to avoid failure due to insufficient wearing time.

An important limitation of this study is the small sample size. However, the similarity of the indexes of periodontal health at baseline between the two experimental groups prompts their comparability and therefore the validity of findings. The only two indices distributed differently in the two experimental groups indicate a worse baseline periodontal situation in the group treated with Invisalign® compared to the group treated with fixed brackets. In this situation, the advantage of treatment with Invisalign® compared to treatment with fixed appliances for periodontal health would rather be underestimated.

Another limitation of the study is that microbiological analysis could be performed only in two sites (molar and incisor) and only four bacteria could be analyzed. This was due to the need to contain costs. However, more extensive microbiological

procedures performed in all the sites of the oral cavity could be advisable. Another limitation is duration of follow-up. Longitudinal data at end of treatment and long-term evolution of periodontal health are not considered in this preliminary study. This could be an interesting field of research for future trials.
Strength can be found in the analysis of the compliance to oral hygiene which actually needs to be classified and scored, representing a precondition to preserve periodontal health during the orthodontic therapy.
This study did not aim to evaluate the orthodontic outcome of two different approaches (fixed and removable) but the periodontal health in a population of teenagers in which both techniques could be performed. This can represent for the clinician a possibility to consider different treatment options, mainly in teenagers in which compliance to oral hygiene can be difficult to obtain and esthetic concern is particularly present.
The results of this preliminary study suggest that teenagers treated with removable appliances show better compliance to oral hygiene, less plaque, and gingival inflammatory reactions than peers with fixed appliances, during 12 months of orthodontic therapy.

Conflicts of interest

None of the authors have any financial or non-financial competing interests to disclose.

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Legends

Tab 1. Comparison of full mouth plaque and bleeding scores for patients treated with Invisalign® and fixed orthodontic appliances at the beginning of treatment and 12 months after.

Tab. 2 Comparison of plaque index and probing depth for patients treated with Invisalign® and fixed orthodontic appliances at the beginning of treatment, 3, 6, and 12 months after.

Tab. 3 Comparison of bleeding on probing for patients treated with Invisalign® and fixed orthodontic appliances at the beginning of treatment, 3, 6, and 12 months after.

Tab. 4 Comparison of compliance to oral hygiene for patients treated with Invisalign® and fixed orthodontic appliances 12 months after the beginning of treatment (n).

Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances.

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Short title: Periodontal health in teenagers: removable aligners and fixed appliances.

Acknowledgements

The authors wish to acknowledge Dr. Federico Migliori (Department of Orthodontics, University of Insubria, Varese Italy) and Dr. Silvia Margherini (Department of Oral Hygiene, University of Insubria, Varese Italy) for their precious contribution in data collection.