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Longitudinal changes in microbiology and clinical periodontal parameters after removal of fixed orthodontic appliances

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SUMMARY The aim of this longitudinal study was to monitor patients' microbiological and clinical parameters from bracket placement up to 3 months post-treatment. Twenty-four patients (10 males and 14 females, aged 14.6 ± 1.0 years) were included in this investigation. Microbiology (sub- and supragingival), periodontal probing depth (PPD), bleeding on probing (BOP), and gingival crevicular fluid (GCF) flow were assessed at baseline (T1), at bracket removal (T2), and 3 months post-treatment (T3). A statistical comparison was made over time and between the banded, bonded, and control sites. Repeated measurements on patients were taken into account by modelling the patients as a random factor. Except for PPD and BOP, values were log-transformed before analysis. Corrections for simultaneous hypothesis testing were performed via simulation.

The results demonstrated that sub- and supragingival colony-forming units ratio (CFU ratio aerobe/anaerobe) decreased significantly (relatively more anaerobes) at T2 compared with T1. Between T2 and T3 no significant increase in CFU ratio was seen, resulting in a significantly lower CFU ratio at T3 compared with T1 for subgingival plaque. The difference concerning supragingival plaque between T3 and T1 was not significant. Clinical parameters PPD, POB, and GCF flow showed a significant increase between T1 and T2. Between T2 and T3 these variables decreased significantly but remained significantly higher than at T1 [except for BOP values at the bonded sites ($P = 0.0646$)]. Placement of fixed orthodontic appliances has an influence both on microbial and clinical periodontal parameters, which were only partly normalized, 3 months following the removal of the appliances.

Introduction

The placement of orthodontic bands and brackets influences plaque growth and maturation (Lee *et al.*, 2005; Gomes *et al.*, 2007; van Gastel *et al.*, 2008). Significant differences in biofilm formation and periodontal reaction between different bracket types and between bonded teeth compared with control teeth have been reported (van Gastel *et al.*, 2007).

Most studies on gingival changes after bracket placement suggest only reversible periodontal changes (Thomson, 2002; Gomes *et al.*, 2007). Others, however, have reported significant attachment loss during orthodontic treatment (Janson *et al.*, 2003). It is therefore still unclear whether or not these changes in periodontal and microbial parameters will normalize after the end of active orthodontic therapy. In only a few retrospective studies has the periodontal situation of orthodontically treated patients and non-treated controls been compared (Gomes *et al.*, 2007). Prospective studies are needed to further investigate this topic. Therefore, the aims of this study were to investigate the microbial and clinical periodontal changes after placement of orthodontic bands and brackets and to determine whether or not these parameters normalize after appliance removal at the end of active orthodontic therapy.

Subjects and methods

Subjects

Twenty-four patients (10 males and 14 females), aged 14.6 ± 1.1 years, referred to the School of Dentistry at Leuven, were included in this study (Table 1). The patients and their parents were given a written explanation on the background of the study, its objectives, and their involvement and were asked to give written informed consent. This study was approved by the Ethics Committee of the Catholic University of Leuven. The patients were selected if they fulfilled the following inclusion criteria: non-smoker, absence of extensive dental restorations or adhesive fixed partial dentures, a sulcus bleeding index (Muhlemann and Son, 1971) of less than 0.3, no pre-existing periodontal disease, and no use of antibiotics during or up to 4 months prior to the start of the study. The patients were asked whether they were right- or left-handed; handedness might lead to

Table 1 General information on study population with data on placement of bands, age, and gender distribution.

			Age (years)	
	N	Headgear/bands	Mean	SD
Male	10	6	14.6	0.7
Female	14	8	14.7	1.4
Total	24	14	14.6	1.1

differences in brushing left or right, resulting in possible differences in gingival health (Addy *et al.*, 1987). All patients were right-handed. Fourteen (six males) of the 24 patients were treated with headgear (headgear group) and received bands on the upper first molars for a period of 18 weeks prior to bonding brackets to the remaining upper teeth. In the headgear group, it was possible to make intra-subject comparisons between the bonded and banded sites. The other 10 patients were treated with brackets only (non-headgear group).

Experimental design

The study had a longitudinal, prospective split-mouth design. Prior to the start of the research, all patients received standardized oral hygiene instruction to ensure a healthy periodontium. Teeth 14 and 16 were sampled for the headgear group, tooth 16 was a banded site and tooth 14 a bonded site. For the non-headgear group both teeth were bonded. During the study period, the subjects were periodontally analysed on three occasions (Table 2). Baseline (T1) was a different point in time for the banded (T-18) and the bonded (T0) teeth. The headgear group was seen first at T-18 to record the status of the periodontium, to sample the sub- and supragingival plaque, and to insert the molar bands. At the second visit after 18 weeks (T0), the measurements and samples were repeated and brackets were bonded on the remaining upper teeth (headgear group). For the non-headgear group, T0 was the first visit. At T0, the initial orthodontic archwire was also inserted. Thus, T-18 is considered baseline (T1) for the banded sites, whereas T0 is considered baseline (T1) for the bonded sites. Just before (T2) and 3 months after (T3) bracket removal, the measurements were again performed. Standardized oral hygiene instruction with an orthodontic toothbrush (Oral-B, Kirkland, Quebec, Canada) using the Bass technique (Heasman *et al.*, 1998) and a single-tufted brush (Oral-B) was provided. Interdental cleaning was recommended with extra fine interdental wooden sticks (Oral-B). The patients were also able to use these wooden sticks after placement of the bands, brackets, and orthodontic wire. The patients were told to always brush their teeth for 3 minutes. The hygiene protocol was explained using a model and afterwards the brushing of the subjects was analysed and improved by a clinician (JvG) in order to achieve good comprehension (Ay *et al.*, 2007). At each visit, the teeth were stained with erythrosine disclosing solution (4% erythrosine in water) to show the patients how to remove the remaining plaque.

Band placement (T-18)

Only the patients from the headgear group received orthodontic bands on their upper first molars (Table 2) at T-18. The teeth were pumiced with a rubber cup, the correct size was selected and the orthodontic bands were fitted. The gingival band margins were trimmed in order to be placed supragingivally. After disinfecting the bands with alcohol and drying, Transbond™ Plus glass ionomer cement (Unitek™ Multi-Cure Ionomer Orthodontic Band Cement; 3M Unitek, Monrovia, California, USA) was mixed according to the manufacturers' instructions. The bands were then placed and any excess cement was removed from the occlusal and cervical margins of the bands and teeth. All band selection and cementation were performed by the same clinician (JvG). The cement was light cured with a QHL75 halogen curing light (Dentsply, Addlestone, Surrey, UK) for 30 seconds from the occlusal side. The preformed headgear was adjusted and the patients were instructed to wear it for 14 hours a day. This point in time was baseline for the banded sites (T1).

Bracket placement (T0)

At T0, all patients received brackets in the upper arch (Table 2). For the non-headgear group, these were the first orthodontic appliances inserted in the mouth. The headgear group received brackets on all the remaining teeth in the upper jaw. The teeth were pumiced by means of a rubber cup and the quadrant to be bonded was isolated with cotton rolls and saliva suction. The one-step adhesive (Transbond™ Plus Self Etching Primer; 3M Unitek) was applied with a microbrush and the excess was blown away with dry air in the incisal/occlusal direction in order to avoid contact with the gingiva. The composite bonding material (Transbond™ Plus colour change adhesive; 3M Unitek) was applied to the bracket base, the bracket was pressed firmly onto the enamel surface, and any excess adhesive was removed with a probe. The composite was then light cured (QHL75 halogen curing light; Dentsply) for 30 seconds from the occlusal and gingival directions. After placement of the brackets, an initial nickel–titanium orthodontic wire (0.014 inch) was inserted and ligated to the brackets by means of elastomeric ligatures. This point in time was baseline for the bonded sites (T1).

Bracket removal (T2)

The brackets were removed after sampling the plaque and measuring the periodontal parameters. After debonding, all adhesive was removed from the teeth with a carbide bur (H282 204 010; Komet, Croydon, Surrey, UK). After drying,

Table 2 Details of the study with the interventions depicted per contact and per group.

	T1							
	T-18		T0		T2		T3	
Interventions	Headgear group	Non-headgear group	Headgear group	Non-headgear group	Headgear group	Non-headgear group	Headgear group	Non-headgear group
Molar band placement	×							
Bracket placement			×	×				
Debonding					×	×		
Crevicular fluid sampling	×			×	×	×	×	×
Probing depth measuring	×			×	×	×	×	×
Bleeding on probing measuring	×			×	×	×	×	×
Supragingival microbial sampling	×			×	×	×	×	×
Subgingival microbial sampling	×			×	×	×	×	×
Oral hygiene instruction	×			×	×	×	×	×
Scaling and polishing	×		×	×			×	×

T1 is baseline (T-18 for the headgear group, T0 for the non-headgear group). T2 is at the day of debonding. T3 is 3 months after debonding and the endpoint of the study.

the teeth were again inspected for remaining adhesive. When the teeth were free of bonding material, they were cleaned with manual and sonic scalers and pumiced.

Three months after bracket removal (T3)

Three months after T2, the subjects were recalled for plaque sampling and to assess the periodontal parameters.

Microbial sampling

After isolating the teeth from saliva with cotton rolls and gently drying them to prevent contamination, the supragingival plaque was carefully removed by means of sterile curettes without traumatizing the gingiva as this would increase the production of gingival crevicular fluid (GCF; Tanaka *et al.*, 1998). The supragingival plaque to be processed was transferred into flip-capped vials containing 2.0 ml pre-reduced transport fluid (RTF) (Syed and Loesche, 1972).

Each sample was homogenized by vortexing for 30 seconds and coded. The coding was not revealed until all analyses were completed, leading to blinded microbiological analyses.

The subgingival plaque was sampled after collecting the GCF in order not to traumatize the crevice. Six sterile medium paper points (RoekoA, Roeko, Langenau, Germany) were inserted per site (three mesially and three distally) and kept in place for at least 10 seconds. The subgingival plaque samples were processed similar to the supragingival samples (van Gastel *et al.*, 2007).

Gingival crevicular fluid

After removing all supragingival plaque (as described above), the GCF was sampled. The absence of plaque is important because dental plaque itself has also been shown to have a marked effect on the recorded volume of GCF in the strip (Stoller *et al.*, 1990; Griffiths *et al.*, 1992; Griffiths, 2003).

The mesiobuccal and distobuccal sites of teeth 14 and 16 were sampled. For the subjects in non-headgear group, both teeth 14 and 16 were bonded teeth and the samples could thereby be pooled. Periopaper® (#593525; Ora Flow Inc., Amityville, New York, USA) absorbent strips were placed into the sulcus until slight resistance was experienced (Griffiths, 2003). After keeping the strip in place for 30 seconds, the absorbed volume was measured with the Periotron® 6000 (Ora Flow Inc.). Strips with signs of blood contamination were discarded. The measurements were performed within 5 seconds after removal of the strip from the crevice to minimize evaporation (Tozum *et al.*, 2004). For each site three strips were used.

Periodontal parameters

At all visits, digital colour photographs were taken in order to archive the status of the periodontium and of the dental plaque accumulation.

Probing depths were measured at the proximal buccal sides of the teeth with a Merrit B® Probe (Hu-Friedy, Chicago, Illinois, USA) and rounded off to the nearest 0.5 mm. Bleeding on probing (BOP) tendency for each of the above-mentioned sites per tooth was also recorded, 20 seconds after probing the depth of the pocket (absent = 0 and present = 1). These parameters were scored at all visits and the examiner was blinded from the previous scores.

Culture techniques

All samples were transferred to the laboratory and processed within 2 hours. Serial 10-fold dilutions were prepared in

RTF. Dilutions of 10^{-2} to 10^{-4} were plated in duplicate by means of a spiral platter (Spiral Systems® Inc., Cincinnati, Ohio, USA) onto non-selective blood agar plates (Blood Agar Base II®; Oxoid, Basingstoke, Hampshire, UK), supplemented with haemine (5 μg/ml), menadione (1 μg/ml), and 5 per cent sterile horse blood.

After 7 days of anaerobic incubation (80 per cent N_2, 10 per cent CO_2, and 10 per cent H_2) in an anaerobic chamber and 3 days of aerobic incubation at 37°C, the total number of anaerobic and aerobic colony-forming units (CFU) were counted. From these data, the CFU ratio (CFUaerobe/CFUanaerobe) was also calculated. The number of specific dark pigmented colonies (black-pigmented bacteria) on a non-selective anaerobic plate, containing approximately 100 colonies, was counted. From the black-pigmented bacteria in the plaque samples, every third colony was subcultured on a blood agar plate. After 48 hours of anaerobic incubation, the pure cultures were identified by means of a series of biochemical tests (including *N*-acetyl-β-D-glucosaminidase, α-glucosidase, α-galactosidase, α-fucosidase, esculine, indole, and trypsin activity) in order to differentiate *Porphyromonas gingivalis* and *Prevotella intermedia* from other pigmented *Porphyromonas* and *Prevotella* species.

Statistical analysis

A linear mixed model was used with the data, using time, type, and their interaction as fixed factors. Repeated measurements on patients were taken into account by modelling the patients as a random factor. Except for periodontal probing depth (PPD) and BOP, the values were log-transformed before analysis. Multiple comparisons between types and times were set up and a comparison of times was also performed for two types of subgroups: pathological or non-pathological pocket depth on the one hand and upper and lower half of the GCF flow at removal of the bands/brackets on the other. Corrections for simultaneous hypothesis testing were performed via simulation.

Results

Microbiology

The banded and bonded sites showed the same tendency concerning supragingival CFU ratio: they decreased significantly between T1 and T2 and between T2 and T3 they increased but not significantly (Figure 1A). Ultimately, the CFU ratio (aerobe/anaerobe) at T3 remained lower (factor 1.7 for the banded and 1.8 for the bonded sites) than at T1, but this difference was not significant. No significant differences between the banded and bonded sites were seen during the study. The presence of *P. intermedia* in the supragingival dental plaque increased significantly from T1 to T2 for the banded sites and then decreased between T2 and T3 to normal values (T3 not significantly different from T1). The subgingival CFU ratio also significantly decreased between T1 and T2 for both the banded and bonded sites. Between T2 and T3 it increased, but not significantly, leading to significantly elevated pathology concerning the subgingival microbiology at T3 compared with T1 for both the bonded and the banded sites. The prevalence of *P. intermedia* in the subgingival plaque showed no significant change over time. When the patients with the higher GCF flows at T2 were analysed, a significant increase in subgingival CFU ratio was seen between T2 and T3 for both the banded and bonded sites. When the results were grouped according to PPD greater or less than 4 mm, no differences were observed.

Periodontal parameters

Gingival crevicular fluid. The banded as well as the bonded sites showed the same tendency: the GCF flow showed significantly elevated levels at T2 compared with T1 (Figure 1B). Three months after debonding (T3), GCF flow was significantly decreased compared with T2 but remained significantly higher than the values at T1. No significant differences between the banded and bonded sites were seen at any of the assessments.

Periodontal probing depth. PPD showed a significant increase between T1 and T2 for both the banded and the bonded sites (Figure 1C). Between T2 and T3 PPD reduced significantly but remained significantly higher than at T1. When grouped according to probing depths greater or less than 4 mm, no differences were observed. No significant differences between the banded and bonded sites were seen for any of the assessments.

Bleeding on probing. The number of proximal sites that showed BOP increased significantly between T1 and T2 for both the banded and the bonded sites (Figure 1D). At T3, the banded sites still showed a significantly higher number of sites with BOP than at T1. For the bonded sites, the difference between T3 and T1 was borderline significant ($P = 0.0646$). When grouped according to probing depths greater or less than 4 mm, no differences were observed. Patients with probing depths less than 4 mm at T2 showed a significant decrease in BOP at T3. No significant differences between the banded and bonded sites were seen at any of the assessments.

Discussion

This prospective study was carried out because microbial and clinical periodontal data after completion of orthodontic treatment are largely lacking. The evaluation time was set at 3 months post-treatment because this is the time at this institute when a decision is made as to whether or not to perform a gingivoplasty in case of gingival hypertrophy after orthodontic treatment.

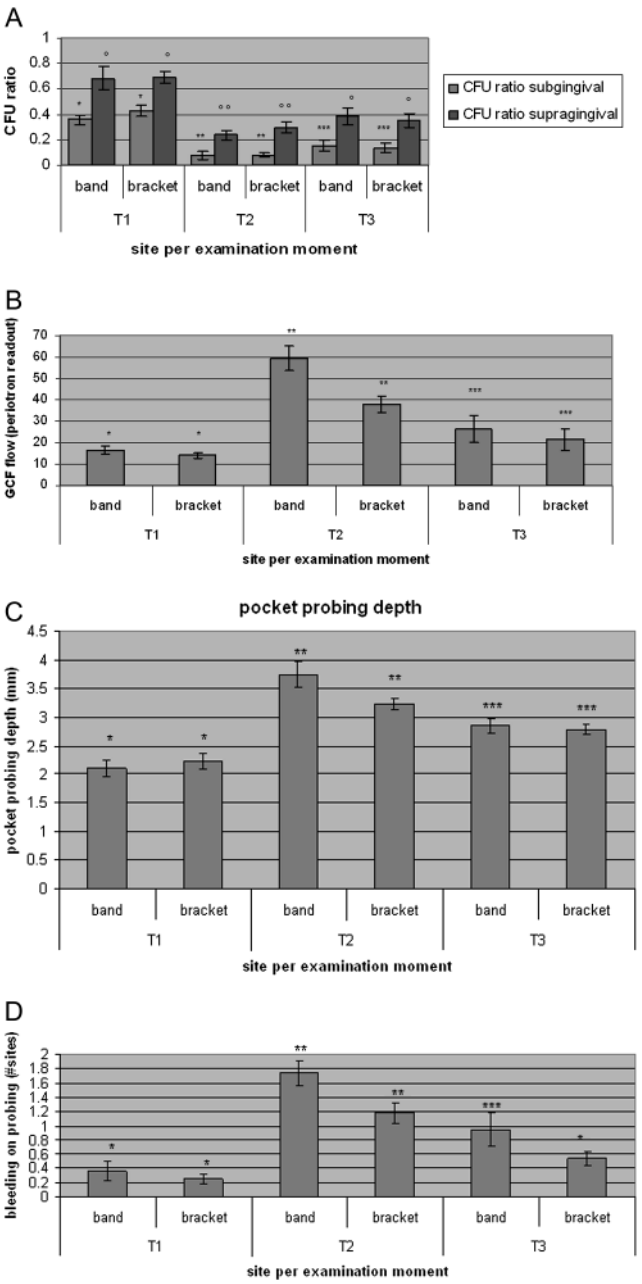

Figure 1 (A) Colony-forming units (CFU) ratio (aerobe/anaerobe), (B) gingival crevicular fluid (GCF) flow (Periotron® readout), (C) probing depth (PPD in mm), and (D) number of sites with bleeding on probing (BOP) for the banded and bonded sites. Values are displayed as the mean and standard deviation at baseline, before the attachments were placed (T1), at bracket removal (T2), and 3 months after bracket removal (T3). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$

It was not possible to measure the periodontal parameters blinded, but the researcher was blinded to the previous scores. All laboratory analyses were performed with an unknown coding system, which was only revealed after completion of the study.

The increased PPD recorded was most likely caused by pseudo-pocket formation or by deeper penetration of the probe into the weakened connective tissue (Jansen *et al.*, 1981; Anderson *et al.*, 1991). As these two processes could simultaneously contribute to the increase in PPD, a distinction could be made with the instruments used in this study. During the period of this experiment, gingivitis was induced but attachment loss probably did not occur (Thomson, 2002; Gomes *et al.*, 2007).

Total removal of the dental plaque at each visit was not possible due to the presence of orthodontic bands and brackets; this could also give rise to trauma to the gingival margin leading to increased GCF flow. For these reasons, the absolute values of the CFU were not considered, and only the CFU ratios were compared. This ratio between aerobic and anaerobic bacteria is an important parameter to score the pathogenicity of plaque (Socransky *et al.*, 1991).

Bacterial culturing has been the classic diagnostic method used to study the composition of dental plaque and is still generally used in periodontal research (Lau *et al.*, 2004; Verner *et al.*, 2006). The main advantages of this method are its capacity to detect multiple bacterial species simultaneously and the possibility to obtain relative and absolute counts of the cultured species. Moreover, it is the only method with which it is possible to detect unexpected bacteria, to correctly characterize new species, and to assess the antibiotic sensitivity of the grown bacteria (Eick and Pfister, 2002; Perinetti *et al.*, 2004). The disadvantages are that the anaerobic culturing procedure recovers only a part of the microscopic count obtained on the same plaque sample (Jervoe-Storm *et al.*, 2005). This difference is usually attributed to the presence of uncultivable organisms, such as the various spirochaetal species, which are not likely to be present in these young patients. The culturing technique relies on the detection of viable organisms and requires that samples are almost immediately processed upon acquisition in order to maximize bacterial survival, in conjunction with essential strict transport conditions (Kamma *et al.*, 2004). While the sensitivity of this method can be rather low, so that small numbers of a specific pathogen in a sample can remain undetected, this was of less importance in this study since the main interest was the overall changes over time (Kamma *et al.*, 2004).

GCF sampling by paper strips may significantly affect subgingival sampling at the same site but this influence will be similar at all sites because the subgingival plaque sampling was always preceded by GCF sampling (Mullally *et al.*, 1994). Collection of the subgingival plaque before GCF sampling was not considered because the traumatizing effect of the paper points could lead to increased GCF flows. A significant decrease in CFU ratio (aerobe/anaerobe) and thus an increase in pathogenicity of the dental plaque was seen between the beginning and end of treatment (Socransky *et al.*, 1991). This alteration in microbial composition has also been described by others (Kloehn and Pfeifer, 1974; Naranjo *et al.*, 2006; van Gastel *et al.*, 2008). The increased supragingival CFU ratio 3 months after debonding did

not differ significantly from that at T1. The subgingival CFU that (aerobe/anaerobe) at T3 on the other hand was significantly different from that at T1. This difference might be explained by the fact that the supragingival microbial composition is strongly influenced by the possibility of improved oral hygiene after debonding.

The increased pathogenicity of the dental plaque and the concomitant periodontal changes during orthodontic treatment have been described by several authors (Petti *et al.*, 1997; Naranjo *et al.*, 2006; van Gastel *et al.*, 2008). Sallum *et al.* (2004) reported on the microbial and periodontal changes such as plaque index, gingivitis index, and PPD after bracket removal. The samples were taken twice: first during the final phase of orthodontic treatment and second 30 days after bracket removal and professional prophylaxis. Those authors concluded that the periodontal signs of gingival inflammation decreased significantly after bracket removal. This improvement in periodontal health 30 days after bracket removal was accompanied by a reduction of the number of sites positive for *Actinobacillus actinomycetemcomitans* and *Bacteroides forsythus*. The initial periodontal status before the start of treatment of the patients in the study of Sallum *et al.* (2004) was not taken into account and thus the conclusions are incomplete.

In a study by Gomes *et al.* (2007), the periodontal conditions of orthodontically treated and untreated individuals were compared. Their results showed similar periodontal conditions for both groups and suggested an absence of permanent periodontal damage, traditionally related to fixed appliances, especially to banded molars (Gomes *et al.*, 2007). Their study population consisted of dental students with good oral hygiene, which makes it difficult to extrapolate these findings to the general orthodontic population.

Recently, Thornberg *et al.* (2009) attempted to document the changes of eight putative periodontal pathogens in patients before, during, and 3 months after fixed orthodontic appliance treatment. They concluded that the percentages of subjects with high pathogen counts increased significantly after 6 months of treatment compared with pre-treatment, then returned to pre-treatment level after 12 months of treatment. No pathogen level was significantly higher after 12 months, and orthodontic treatment was found to be significantly protective for half of the periodontal pathogens (*Fusobacterium nucleatum, Eikenella corrodens, Treponema denticola,* and *Campylobacter rectus*; Thornberg *et al.*, 2009). These data are contrary to the present microbial results.

Conclusion

Placement of fixed orthodontic appliances has a significant impact on microbial and clinical periodontal parameters. All values were significantly increased at bracket removal (T2) compared with T1. The supragingival CFU ratio normalized after 3 months, possibly because these sites are more sensitive to changes in oral hygiene. The subgingival CFU ratio 3 months after bracket removal (T3) remained significantly lower compared with T1, indicating that the changes induced by orthodontic treatment are partially irreversible. The periodontal values tended to normalize after debonding, but most values remained significantly elevated at T3 compared with T1.

It would be interesting to repeat these measurements after a longer period of time to elucidate long-term changes. If no further changes occur, it would be justifiable to carry out further periodontal treatment 3 months after treatment to reduce PPD.

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