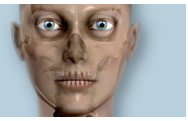
Orthodontics & Craniofacial Research



ORIGINAL ARTICLE

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Long-term changes in microbiology and clinical periodontal variables after completion of fixed orthodontic appliances

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Structured Abstract

Objectives – The aim of this prospective study was to monitor patients' microbiological and clinical periodontal parameters prior and up to 2 years after orthodontic treatment.

Material and Methods – Twenty-four adolescents were treated with brackets. Fourteen of them received bands on upper first molars for extra-oral force application before bonding brackets to the remaining teeth. Microbiology, periodontal probing depth, bleeding on probing (BOP), and gingival crevicular fluid (GCF) flow were assessed at baseline (T1), bracket removal (T2), and 2 years post-treatment (T3). A statistical comparison was made over time and between bands and brackets.

Results – A significant increase from T1 to T2 and a decrease from T2 to T3 in pathogenicity of plaque were noted. No significant difference was observed concerning supragingival colony-forming units (CFU) ratio (aerobe/anaerobe) between T3 and T1. However, the subgingival CFU ratio (aerobe/anaerobe) at T3 did significantly differ from the ratio at T1. Periodontal probing depth, BOP and GCF flow showed a significant increase between T1 and T2 and a reduction between T2 and T3, resulting in the absence of significant differences between T3 and T1, except for BOP at banded sites.

Conclusion – Placement of fixed appliances has an impact on periodontal parameters. The results showed that not all parameters were normalized at T3, indicating that the changes are only partially reversible.

Key words: fixed appliance; orthodontics; periodontal health; subgingival microflora; supragingival microflora

Introduction

Fixed orthodontic treatment is the method of choice in contemporary orthodontics. The placement of orthodontic bands and brackets may compromise an optimal oral hygiene, resulting in accumulation and maturation of dental plaque (1–4). It is well established that bacterial plaque is the primary etiological factor in the development of gingival inflammation and periodontitis (5–7).

A systematic review on the effects of orthodontic treatment on periodontal health identified an absence of reliable evidence on this topic. The existing low-quality evidence suggests that orthodontic treatment results in small detrimental effects to the periodontium. The relative short-term follow-up does not allow to extrapolate the long-term effects of orthodontic appliances on periodontal parameters (8, 9).

In a previous study, it was reported that the clinical periodontal values (PPD, BOP and GCF flow) and microbiology tended to normalize after debonding, but most values remained significantly elevated 3 months after debonding compared with baseline (10). As the periodontal parameters only partly normalized 3 months after debonding, repeating these measurements after a longer period of time was recommended to elucidate long-term changes.

Therefore, the aims of this longitudinal prospective study were to investigate microbial and clinical periodontal changes after placement of orthodontic bands and brackets and to determine whether or not these parameters further normalize 2 years after termination of the orthodontic treatment by means of fixed appliances.

Material and methods

Subjects

Twenty-four patients (10 males, 14 females), aged 14.6 ± 1.1 years, referred to the Department of Orthodontics of the University Hospital Leuven, were included. All patients fulfilled the following inclusion criteria: no systemic disease, non-smoker, absence of extensive dental restorations, or adhesive fixed partial dentures, a sulcus bleeding index <0.3 (11); no pre-existing periodontal disease and no use of antibiotics during or up to 4 months prior to the study. The patients and their parents all gave written informed consent. This study has been reviewed and approved by the Ethics Committee of the Katholieke Universiteit Leuven. Ten patients (four males, six females) were treated with brackets only (non-headgear group). The other 14 subjects were additionally treated with a headgear (headgear group). Forces of 150–200 gram per side were used to derotate the first molars to establish and keep a solid neutro-occlusion of the first molars. Patients were instructed to wear the cervical headgear only during nighttime.

Experimental design

The study had a longitudinal prospective design and is a continuation of the study of van Gastel et al. (10). This study includes subjects about which van Gastel et al. (10) have published a previous article in which data at baseline (T1), bracket removal (T2), and 3 months post-treatment were presented. Data from 2 years after bracket removal were added in this study.

At teeth, 14 and 16 samples and periodontal measurements were performed. Patients were told not to eat or drink 2 h before their appointment. The patients were instructed not to brush their teeth in the morning, and all patients were seen between 10 and 12 a.m. For the headgear group, tooth 16 was a banded site and tooth 14 was a bonded site because only the patients from this group received bands on their upper first molars. The subjects were periodontally checked at four occasions (Table 1). Baseline (T1) was a different point in time for banded (T-18) and bonded (T0) teeth. The headgear group was first seen at T-18 to insert the molar bands. After 18 weeks, brackets were bonded on the remaining teeth (T0). For the non-headgear group, T0 was the first visit. Thereby, T-18 is considered baseline (T1) for the headgear group, whereas T0 is considered baseline (T1) for the non-headgear group. Just before bracket removal

Table 1. **Details on 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	x							
Bracket placement			x	x				
Debonding					x	x		
Crevicular fluid sampling	x			x	x	x	x	x
Probing depth measuring	x			x	x	x	x	x
Bleeding on probing measuring	x			x	x	x	x	x
Supragingival microbial sampling	x			x	x	x	x	x
Subgingival microbial sampling	x			x	x	x	x	x
Oral hygiene instruction	x			x	x	x		
Scaling and polishing	x		x	x				

T1 is baseline (T-18 for the headgear group, T0 for the non-headgear group). T2 is at debonding. T3 is 2 years after debonding and the endpoint of this study.

(T2) and 2 years (T3) after removal, the measurements were repeated. Only 21 subjects were analyzed at T3, as one subject of the headgear group and two of the non-headgear group were not traceable. The active orthodontic treatment duration was 21 ± 3 months (mean $\pm$ SD). Afterward, fixed retainers were bonded as far from the gingival tissue as the established occlusion allowed on the palatal/lingual surfaces of the six upper and lower anterior teeth.

Scaling and polishing were carried out after performing the microbial and periodontal sampling at baseline (T1) and 3 months after debonding (Table 1). Scaling and pumicing consisted of supra- and subgingival removal of plaque and calculus with manual and ultrasonic scalers and polishing with a rubber cup and pumice.

Intra-subject comparisons were made over time and between banded and bonded sites. Differences of the investigated parameters between banded and bonded sites and differences of these parameters between T1, T2, and T3 were studied. The comparisons were made for all data and after grouping them according to pathological (PPD >4 mm) or non-pathological (PPD ≤4 mm) pocket depth and upper or lower half of the GCF flow at removal of the bands/brackets at T2. The PPD results were grouped, because we assumed that a PPD value of more than 4 mm would make cleaning more difficult, and therefore, the reduction in PPD less pronounced. The GCF flow results were grouped because this is a very accurate and direct parameter of inflammation, with a higher distinctive value than BOP for instance. To group according to GCF flow, all sites were ranked from high to low scores for GCF flow at T2. Subsequently, the sites were divided into two even groups: sites with high values for GCF flow and sites with low values for GCF flow. Sites in the group of high values for GCF flow were defined as 'sites with upper half of the GCF flow at T2'. Sites in the other group with low values for GCF flow were

defined as 'sites with lower half of the GCF flow at T2'. These divisions were made to check whether the values for sites with pathological PPD (PPD >4 mm) or sites with upper half of GCF flow at T2 differ more or less between T3 on the one hand and T1 and T2 on the other hand than the other sites. Also, a difference between bands and brackets with pathological PPD (PPD >4 mm) or sites with upper half of GCF flow and between band and brackets with non-pathological PPD (PPD ≤4 mm) or sites with lower half of GCF flow could be investigated.

Microbiological parameters

After isolating the teeth from saliva, the supragingival plaque was removed by means of sterile curettes. The subgingival plaque was sampled after collecting the GCF. Six sterile medium paper points (RoekoA, Roeko, Langenau, Germany) were inserted per site (three mesially and three distally) and kept in place for 10 s. The sub- and supragingival plaque was transferred into flip-capped vials containing 2.0 ml prereduced transport medium (RTF) to be processed (12, 13). Each sample was homogenized by vortexing for 30 s. Serial 10-fold dilutions were prepared in RTF. Dilutions of 10^{-1} to 10^{-4} were plated by means of a spiral platter (Spiral Systems®, Inc. Cincinnati, OH, USA) onto non-selective blood agar plates (Blood Agar Base II®, Oxoid, Basingstoke, Hampshire, UK), supplemented with haemin (5 μg/ml), menadione (1 μg/ml) and 5% sterile horse blood.

After 7 days of anaerobic incubation in an anaerobic jar system (10% CO_2, 10% H_2 and 80% N_2) at 37°C (14, 15) and 3 days of aerobic incubation at 37°C (16), the total number of respectively anaerobic and aerobic colony-forming units (CFU) were counted. From this data, the CFU ratio (CFUaerobe/CFUanaerobe) was calculated. The number of specific black-pigmented colonies on a non-selective anaerobic plate, containing approximately 100 colonies, was counted.

Periodontal parameters

Probing depths were measured at the proximal buccal sides of the teeth 14 and 16 with a Merrit B® Probe (Hu-friedy, Chicago, IL, USA) and rounded off to the nearest 0.5 mm. After 20 s, bleeding on probing tendency (BOP) for each of the above-mentioned sites per tooth was recorded (absent = 0, present = 1).

After removing all supragingival plaque, the GCF was sampled. The absence of dental plaque is important because plaque itself has also been shown to have an effect on the recorded volume of GCF in the strip (17–19). The mesiobuccal and distobuccal sites of the teeth 14 and 16 were sampled. Periopaper® (#593525, Ora Flow Inc., Amityville, NY, USA) strips were placed into the sulcus until slight resistance was experienced (20). After keeping the strip in place for 30 s, the absorbed volume was measured with the Periotron® 6000 (Ora Flow Inc., PlainView, NY, USA).

Statistical analysis

A linear mixed model was used with the data using time, type (bonded or banded sites), and their interaction as fixed factors. Repeated measurements on patients were taken into account by modeling the patients as a random factor. Except for 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 (PPD >4 mm) or non-pathological (PPD ≤4 mm) pocket depth on the one hand and upper and lower half of the GCF flow at removal of the bands/brackets on the other hand. Corrections for simultaneous hypothesis testing were performed according to Sidak (21), yielding a significance level of 95% for each set of comparisons.

Results

Microbiology

Supragingival

During the orthodontic treatment, the supragingival CFU ratio (aerobe/anaerobe) decreased significantly between T1 and T2 ($p < 0.05$) for both banded and bonded sites (Fig. 1A). For the banded and bonded sites, the supragingival CFU

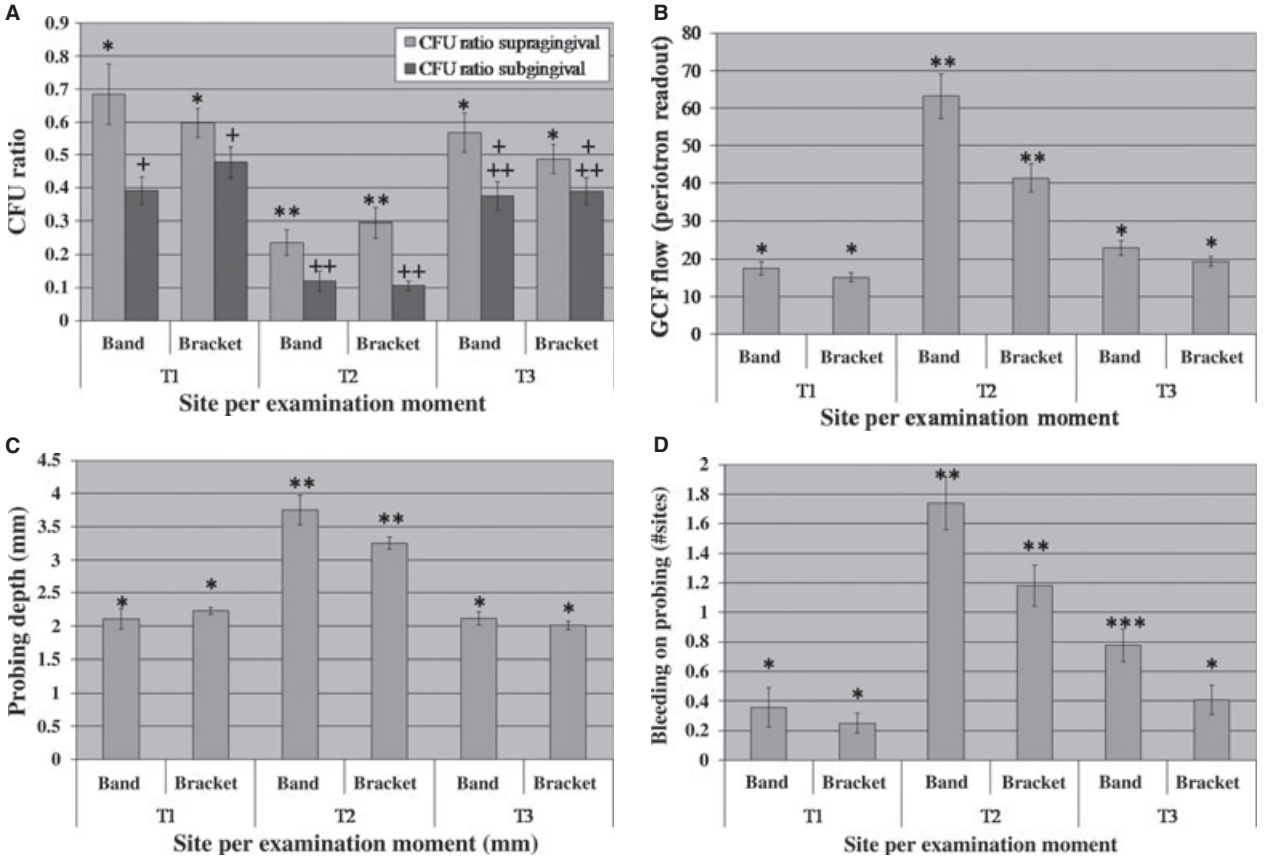

Fig. 1. (A) colony-forming units (CFU) ratio (aerobe/anaerobe), (B) gingival crevicular fluid (GCF) flow (Periotron® readout), (C) periodontal probing depth (PPD) (in mm), and (D) number of sites with bleeding on probing (BOP) for banded and bonded sites. Values are displayed as the mean and standard deviation of the mean at T1, T2, and T3. * vs. ** vs. *** and $^{+}$ vs. $^{++}$ vs. $^{+++}$ indicate significant differences ($p < 0.05$).

ratio increased significantly between T2 and T3, resulting in the absence of a significant difference in the CFU ratio between T3 and T1. When the subjects were grouped according to probing depths either PPD >4 mm or PPD ≤4 mm at T2, only subjects with probing depths lower than 4 mm at T2 showed a statistical significant increase in supragingival CFU ratio (aerobe/anaerobe) between T2 and T3 for both the banded and bonded sites. No significant difference in supragingival CFU ratio between the banded and bonded sites was seen during this study at any of the assessments. The presence of black-pigmented bacteria in the supragingival plaque increased significantly from T1 to T2 for the banded sites ($p < 0.05$). The occurrence of black-pigmented bacteria decreased between T2 and T3 to normal values, leading to no significant difference between T3 and T1. No significant change over time was observed concerning the prevalence of black-pigmented bacteria in the supragingival plaque at bonded sites.

Subgingival

The subgingival CFU ratio (aerobe/anaerobe) also decreased significantly between T1 and T2 for banded and bonded sites ($p < 0.05$). Between T2 and T3, the subgingival CFU ratio increased significantly, leading to significantly elevated pathology for periodontal disease, including gingivitis (22, 23), concerning the subgingival microbiology at T3 compared with T1 for both banded and bonded sites ($p < 0.05$). No significant difference between banded and bonded sites at any assessment was seen during this study. The prevalence of black-pigmented bacteria in the subgingival plaque showed no significant change over time. When all values of

different time assessments were pooled (statistically valid due to the lack of an interaction effect between time and type (banded or bonded sites)), significant higher scores for black-pigmented bacteria were found at banded sites compared with bonded sites.

Periodontal parameters

Gingival crevicular fluid

Banded and bonded sites showed the same tendency: the GCF flow showed significant elevated levels at T2 compared with T1 ($p < 0.05$) (Fig. 1B). Two years after debonding (T3), the GCF flow decreased significantly compared with T2 ($p < 0.05$), and no significant difference was seen between T3 and T1. No significant difference between banded and bonded sites was seen at any of the assessments. Due to the lack of interaction between time and type (banded or bonded sites), all values for banded and bonded sites at different time assessments could be pooled. A significant difference was seen between banded and bonded sites, with the GCF flow being higher for banded sites.

Periodontal probing depth

Periodontal probing depth showed a significant increase between T1 and T2 for both banded and bonded sites ($p < 0.05$) (Fig. 1C). Between T2 and T3, the PPD diminished significantly, resulting in the absence of a significant difference between T3 and T1. When grouped according to probing depths greater or <4 mm at T2, no difference was observed. The same is true when grouped according to upper and lower half of the GCF flow at T2. No significant difference between banded and bonded sites was seen at any of the assessments.

Bleeding on probing

The number of proximal sites that showed BOP increased significantly between T1 and T2 for both banded and bonded sites ($p < 0.05$) (Fig. 1D). The number of sites with BOP for banded sites decreased significantly between T2 (mean 1.738, 95% confidence interval (CI) 1.381–2.095) and T3 (mean 0.794, 95% CI 0.424–1.165), resulting in a significant difference between T3 and T1 (mean 0.357, 95% CI 0–0.714). Bonded sites showed the same tendency as banded sites, but no significant difference was seen between T3 (mean 0.396, 95% CI 0.128–0.664) and T1 (mean 0.248, 95% CI 0–0.504) for bonded sites. Subjects with probing depths even or <4 mm at T2 did show a significant decrease in BOP between T2 and T3 ($p < 0.05$). For this subgroup, a difference between banded and bonded sites was seen at T3: the number of sites with BOP was significantly higher for banded sites. Such significant decrease in BOP between T2 and T3 was not seen in subjects with probing depths higher than 4 mm on T2.

Discussion

This prospective study was carried out because periodontal data after completion of orthodontic treatment are largely lacking. In a previous study, it was reported that the periodontal values tended to normalize after debonding (10). As the periodontal parameters only partly normalized 3 months after debonding, repeating these measurements after a longer period of time was recommended to elucidate long-term changes. The evaluation time was set at 2 years post-treatment.

Total removal of dental plaque at each visit was not possible due to the presence of orthodontic appliances and the risk of trauma of the gingiva. Therefore, only CFU ratios (aerobe/anaerobe), which is an important parameter to score the pathogenicity of plaque, were analyzed (22–24).

Bacterial culturing has been the classic diagnostic method used in the study of the composition of plaque and is still often used in periodontal research (24–26). 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 cultured species. Moreover, it is the method of choice to detect unexpected bacteria, to correctly characterize new species, and to assess the antibiotic sensitivity of the grown bacteria (27, 28). Disadvantages are that the anaerobic

culturing procedure recovers only a part of the microscopic count obtained on the same plaque sample (29). This difference is usually attributed to the presence of uncultivable organisms, such as various spirochetal species, which are not likely to be present in the young patients of this study (30, 31). The culturing technique relies on the detection of viable organisms and requires that samples are almost immediately processed upon acquisition to maximize bacterial survival, in conjunction with essential strict transport conditions (32). Furthermore, 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 because the main interest was the overall changes over time (32).

van Gastel et al. (10) reported that the differences concerning the supragingival CFU ratio (aerobe/anaerobe) and the presence of black-pigmented bacteria between 3 months after debonding and baseline were already not statistically significant. The results of this study showed that further normalization occurred. The subgingival CFU ratio (aerobe/anaerobe) increased over time after debonding (T2), but remained significantly different at T3 (2 years after debonding) compared with T1 (baseline). This difference might be explained by the fact that the supragingival microbial composition is strongly influenced by the possibility of improved oral hygiene post-treatment. Concerning PPD and GCF, no significant difference could be seen between T3 and T1. 3 months after debonding, both banded and bonded sites showed a significant higher number with BOP than at baseline. This difference in BOP was normalized 2 years post-treatment compared with pre-treatment values, but only for bonded sites.

Most subjects undergoing orthodontic therapy develop generalized gingivitis within a short time probably associated with the plaque retentive effect of the appliances (1, 3, 4, 33–36). The increased PPD recorded during this study is most likely caused by gingival enlargement or by deeper penetration of the probe into weakened connective tissue (6, 33, 37–40). A significant decrease in CFU ratio (aerobe/anaerobe) and thus an increase in pathogenicity of the plaque were seen between the commencement and end of treatment (22–24). This change in microbial composition has also been reported by other authors (2, 7, 41). The qualitative change in the microbiota, which involves the growth of periodontopathogens, could be associated with the gingival inflammation around orthodontic appliances (6).

Several studies report on the changes in periodontal parameters during orthodontic treatment (1–3, 28, 35, 42–45). The accumulation and increased pathogenicity of plaque during orthodontics are described by several authors (1–3, 28, 35, 42, 43, 45). However, less studies report about the (long-term) clinical and microbiological changes after removal of the appliances (4, 6, 33, 44–48).

Thornberg et al. (48) investigated the changes of eight putative periodontal pathogens in patients before, during, and 3 months after fixed appliances. Their results indicated that subjects with high pathogen counts increased significantly after 6 months of treatment compared with pre-treatment. After 12 months of treatment, the values returned to pre-treatment level. No pathogen level was significantly higher after 12 months of treatment, and orthodontic treatment was found to be significantly protective for half of the periodontal pathogens (48). These data are in contrast to the present results, because we found that the subgingival CFU ratio (aerobe/anaerobe) at T3 did not return to baseline levels.

Recently, Liu et al. (4) reported on the periodontal and microbiological changes during fixed appliances in two groups. One group was examined from prior up to 3 months of orthodontic treatment. The other group was followed from just before removal of the fixed appliances to 6 months post-treatment. Their results showed that a significant increase in plaque index and gingivitis index during the first 3 months of treatment occurred. No such an increase could be seen for the PPD, which might be due to the short evaluation term. Those changes of clinical parameters are in agreement with Naranjo et al. (2). In our earlier publication

by van Gastel et al. (10), it also was described that the clinical parameters PPD, BOP and GCF flow showed a significant increase between T1 and T2. According to Liu et al. (4), the clinical parameters and the carriage and amount of subgingival *Porphyromonas gingivalis* decreased significantly during the 6 months after debonding. Ultimately, the amount of *P. gingivalis* remained higher than before start of the treatment, which may imply a potential risk to periodontal health in some patients. These results are in agreement with the findings of van Gastel et al. (10), in which it also was reported that the clinical parameters decreased after debonding. Concerning the subgingival CFU ratio, van Gastel et al. (10) found an increase between debonding and 3 months post-treatment, resulting in a significant difference between baseline and 3 months post-treatment (10). The present results showed that the subgingival CFU ratio 2 years post-treatment still did significantly differ from the ratio at T1. So both studies, Liu et al. (4) and our results, showed that the values for, respectively, carriage and amount of *P. gingivalis* and subgingival CFU ratio did not return to baseline levels, respectively, 6 months and 2 years after debonding. A disadvantage of the study of Liu et al. (4) is the comparison between pretreatment values of one group and post-treatment values of the other group.

A study conducted by Renkema et al. (45) described the extent of the gingival enlargement in patients during and after treatment with fixed appliances. The gingiva was scored using a visual analog scale (VAS) before placement of the appliances, after removal and 3 and 6 months after debonding. The authors reported that the average degree of gingival enlargement increased significantly during treatment. After debonding, a significant decrease was observed, resulting in the absence of a significant difference in the degree of gingival enlargement between 3 months post-treatment and baseline. The results of our earlier publication van Gastel et al. (10) indicated that 3 months after debonding, the pocket depth remained significantly higher than at baseline. However, between 3 months after debonding and 2 years post-treatment, PPD decreased leading to no statistically significant difference between 2 years post-treatment and baseline. Therefore, the data of Renkema et al. (45) seemed to be contradictory to our results, but VAS is not designed to detect small (significant) differences. So it could be that Renkema et al. (45) could not detect differences 3 months post-treatment compared with baseline due to their inaccurate detection method, although there still might be some small differences between 3 months post-treatment and pretreatment which we could detect. We used the pocket depth to score gingival enlargement and rounded off to the nearest 0.5 mm, which is more precise and can detect smaller differences.

The study of Renkema et al. (45) is in disagreement with a study performed by Kouraki et al. (44) in which 30 subjects with clinically significant gingival enlargement were analyzed at three timepoints: prior to treatment (T1), at bracket removal (T2) and 3–12 months after debonding (T3). The results of the study of Kouraki et al. (44) indicated incomplete resolution of the gingival enlargement. Disadvantages of the study include the method to measure the gingival enlargement and the large spread at T3 between the different subjects, which makes comparison with this study difficult.

No studies were found in the current literature concerning the long-term potential effects of extra-oral force on periodontal tissues. Our study is the first that compared the longitudinal changes in periodontal parameters of bonded sites with banded sites plus a relative low extra-oral force for the night. The results of our study revealed that bonded sites and banded sites plus a low extra-oral force showed the same tendency, except for BOP. Former studies of van Gastel et al. (7, 10), in which the same subjects were used as in this study, revealed that the short-term effects of the banded plus extra-oral force group were comparable to the bonded group, except for BOP (10). This combined with the similar microbial and clinical periodontal reaction of the bonded and banded plus extra-oral force sites during treatment does indicate that the extra-oral force did not have an addi-

tional effect (7). To elucidate the effects of extra-oral force on periodontal health, it would have been interesting to compare the banded sites plus extra-oral force to a control group of banded sites without extra-oral force. Further research on this topic is necessary.

Conclusion

From the data presented in this study, we can conclude that placement of fixed appliances was associated with a deterioration of periodontal parameters; that is, all values were significantly increased at T2 compared with T1. After debonding, all clinical variables, except BOP at banded sites plus extra-oral force, decreased toward levels comparable to values at T1. So the placement of fixed appliances has no long-term impact on the clinical periodontal parameters, because all bonded sites did not have significant differences in clinical periodontal parameters between T3 and T1, except for BOP at banded sites plus extra-oral force. The supragingival CFU ratio (aerobe/anaerobe) also normalized after 2 years. The subgingival CFU ratio (aerobe/anaerobe) at T3 on the other hand was still significantly lower than the ratio at T1. This difference might be explained by the fact that the supragingival microbial composition is more sensitive to changes in improved oral hygiene after debonding.

The results of this study showed that a further normalization toward the values at baseline was seen 2 years after removal of appliances. As further changes occurred between 3 months and 2 years after debonding, it would not be justifiable to carry out further periodontal treatment like a gingivectomy and gingivoplasty to reduce PPD and to improve the gingival contour 3 months after completion of orthodontic therapy. As a conclusion, the results of this study indicate that changes of periodontal parameters associated with fixed orthodontic appliances are, 2 years after appliance removal, only partially reversed.

Clinical relevance

It has been published before that placement of brackets has a negative influence on the periodontium. This study was set up to analyze whether these changes in microbiological and periodontal parameters are reversible.

The results of this study showed that a further normalization toward the values at baseline was seen 2 years after removal of appliances and that the changes induced by orthodontic therapy are only partially reversible. As further changes occurred between 3 months and 2 years after debonding, it would not be justifiable to carry out a gingivectomy or gingivoplasty 3 months after completion of orthodontic therapy.

References

1. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. *Am J Orthod Dentofacial Orthop* 1991;100:337–40.
2. Naranjo AA, Trivino ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. *Am J Orthod Dentofacial Orthop* 2006;130:275.
3. Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents. *Orthod Craniofac Res* 2007;10:187–95.
4. Liu H, Sun J, Dong Y, Lu H, Zhou H, Hansen BF et al. Periodontal health and relative quantity of subgingival Porphyromonas gingivalis during orthodontic treatment. *Angle Orthod* 2011;81:609–15.
5. Löe H. Physiology of the gingival pocket. *Acad Rev Calif Acad Periodontol* 1965;13:6–14.
6. Sallum EJ, Nouer DF, Klein MI, Gonçalves RB, Machion L, Sallum AW et al. Clinical and microbiological changes after removal of orthodontic appliances. *Am J Orthod Dentofacial Orthop* 2004;126:363–6.
7. van Gastel J, Quirynen M, Teughels W, Coucke W, Carels C. Longitudinal changes in microbiology and clinical periodontal variables after placement of fixed orthodontic appliances. *J Periodontol* 2008;79:2078–86.
8. Bollen AM. Effects of malocclusions and orthodontics on periodontal health: evidence from a systematic review. *J Dent Educ* 2008;72:912–8.
9. Bollen AM, Cunha-Cruz J, Bakko DW, Huang GJ, Hujoel PP. The effects of orthodontic therapy on periodontal health: a systematic review of controlled evidence. *J Am Dent Assoc* 2008;139:413–22.
10. van Gastel J, Quirynen M, Teughels W, Coucke W, Carels C. Longitudinal

changes in microbiology and clinical parameters after removal of fixed orthodontic appliances. *Eur J Orthod* 2011;33:15–21.
11. Mühlemann HR, Son S. Gingival sulcus bleeding index: a leading symptom in initial gingivitis. *Helv Odontol Acta* 1971;15:107–13.
12. van Gastel J, Quirynen M, Teughels W, Coucke W, Carels C. Influence of bracket design on microbial and periodontal parameters *in vivo*. *J Clin Periodontol* 2007;34:423–31.
13. Syed SA, Loesche WJ. Survival of human dental plaque flora in various transport media. *Appl Microbiol* 1972;24:638–44.
14. Lopez NJ, Mellado JC, Giglio MS, Leighton GX. Occurrence of certain bacterial species and morphotypes in juvenile periodontitis in Chile. *J Periodontol* 1995;66:559–67.
15. Gajardo M, Silva N, Gomez L, Leon R, Parra B, Contreras A et al. Prevalence of periodontopathic bacteria in aggressive periodontitis patients in a Chilean population. *J Periodontol* 2005;76:289–94.
16. Quirynen M, De Soete M, Pauwels M, Gizani S, Van Meerbeek B, van Steenberghe D. Can toothpaste or tootbrush with antibacterial tufts prevent tootbrush contamination? *J Periodontol* 2003;74:312–22.
17. Stoller NH, Karras DC, Johnson LR. Reliability of crevicular fluid measurements taken in the presence of supragingival plaque. *J Periodontol* 1990;61:670–3.
18. Griffiths GS, Wilton JM, Curtis MA. Contamination of human gingival crevicular fluid by plaque and saliva. *Arch Oral Biol* 1992;37:559–64.
19. Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontol 2000* 2003;31:32–42.
20. Tözüm TF, Hatipoglu H, Yamalik N, Gürsel M, Alptekin NO, Ataglu T et al. Critical steps in electronic volume quantification of gingival crevicular fluid: the potential impact of evaporation, fluid retention, local conditions and repeated measurements. *J Periodontal Res* 2004;39:344–57.
21. Šidák Z. Rectangular confidence regions for the means of multivariate normal distributions. *J Am Statist Assoc* 1967;62:626–33.
22. Lee SM, Yoo SY, Kim HS, Kim KW, Yoon YJ, Lim SH et al. Prevalence of putative periodontopathogens in subgingival dental plaques from gingivitis lesions in Korean orthodontic patients. *J Microbiol* 2005;43:260–5.
23. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J Periodontol* 1992;63:322–31.
24. Socransky SS, Haffajee AD, Smith C, Dibart S. Relation of counts of microbial species to clinical status at the sampled site. *J Clin Periodontol* 1991;18:766–75.
25. Verner C, Lemaitre P, Daniel A, Giumelli B, Lakhssassi N, Sixou M. Carpegen real-time polymerase chain reaction vs. anaerobic culture for periodontal pathogen identification. *Oral Microbiol Immunol* 2006;21:341–6.
26. Atieh MA. Accuracy of real-time polymerase chain reaction versus anaerobic culture in detection of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis: a meta-analysis. *J Periodontol* 2008;79:1620–9.
27. Eick S, Pfister W. Comparison of microbial cultivation and a commercial PCR based method for detection of periodontopathogenic species in subgingival plaque samples. *J Clin Periodontol* 2002;29:638–44.
28. Perinetti G, Paolantonia M, Cordella C, D'Ercole S, Serra E, Piccolomini R. Clinical and microbiological effects of subgingival administration of two active gels on persistent pockets of chronic periodontitis patients. *J Clin Periodontol* 2004;31:273–81.
29. Jervoe-Storm PM, Koltzscher M, Falk W, Dorfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. *J Clin Periodontol* 2005;32:778–83.
30. Barron SL, Riviere GR, Simonson LG, Lukehart SA, Tira DE, O'Neil DW. Use of monoclonal antibodies to enumerate spirochetes and identify Treponema denticola in dental plaque of children, adolescents and young adults. *Oral Microbiol Immunol* 1991;6:97–101.
31. Andrucioli MC, Nelson-Filho P, Matusmoto MA, Saraiva MC, Feres M, de Figueiredo LC et al. Molecular detection of *in-vivo* microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. *Am J Orthod Dentofacial Orthop* 2012;141:24–9.
32. Kamma JJ, Nakou M, Gmur R, Baehni PC. Microbiological profile of early onset/aggressive periodontitis patients. *Oral Microbiol Immunol* 2004;19:314–21.
33. Gomes SC, Varela CC, Da Veiga SL, Rosing CK, Oppermann RV. Periodontal conditions in subjects following orthodontic therapy. A preliminary study. *Eur J Orthod* 2007;29:477–81.
34. Mullaly B, Wolff L, Hardie N, Aeppli D, Pihlstrom B. Effect of gingival fluid collection on subgingival plaque sampling. *J Dent* 1994;22:223–8.
35. Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Effects of fixed orthodontic appliances on subgingival flora. *Int J Dent Hyg* 2008;6:129–36.
36. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. *Eur J Orthod* 2001;23:475–84.
37. Jansen J, Pilot T, Corba N. Histologic evaluation of probe penetration during clinical assessment of periodontal attachment levels. An investigation of experimentally induced periodontal lesions in beagle dogs. *J Clin Periodontol* 1981;8:98–106.
38. Anderson GB, Cafesse RG, Nasjleti CE, Smith BA. Correlation of periodontal probe penetration and degree of inflammation. *Am J Dent* 1991;4:177–83.
39. Thomson WM. Orthodontic treatment outcomes in the long term: findings from a longitudinal study of New Zealanders. *Angle Orthod* 2002;72:449–55.
40. Amasyali M, Enhos S, Uysal T, Saygun I, Kilic A, Bedir O. Effect of a self-etching adhesive containing an antibacterial monomer on clinical periodontal parameters and subgingival microbiologic composition in orthodontic patients. *Am J Orthod Dentofacial Orthop* 2011;140:e147–53.
41. Kloehn JS, Pfeifer JS. The effect of orthodontic treatment on the periodontium. *Angle Orthod* 1974;44:127–34.

42. Diamanti-Kipioti A, Gusberti FA, Lang NP. Clinical and microbiological effects of fixed orthodontic appliances. *J Clin Periodontol* 1987;14:326–33.
43. Petti S, Barbato E, Simonetti DA. Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. *New Microbiol* 1997;20:55–62.
44. Kouraki E, Bissadi NF, Palomo JM, Ficara AJ. Gingival enlargement and resolution during and after orthodontic treatment. *NY State Dent J* 2005;71:34–7.
45. Renkema AAP, Dusseldorp JK, Middel B, Ren Y. Zwelling van de gingival bij orthodontische behandeling met volledig vaste apparatuur. *Ned Tijdschr Tandheelkd* 2010;117:507–12.
46. Sadowsky C, BeGole EA. Long-term effects of orthodontic treatment on periodontal health. *Am J Orthod* 1981;80:156–72.
47. Polson AM, Subtelny JD, Meitner SW, Polson AP, Sommers EW, Iker HP et al. Long-term periodontal status after orthodontic treatment. *Am J Orthod Dentofacial Orthop* 1988;93:51–8.
48. Thornberg MJ, Riolo CS, Bayirli B, Riolo ML, Van Tubergen EA, Kulbersh R. Periodontal pathogen levels in adolescents before, during and after fixed orthodontic appliance therapy. *Am J Orthod Dentofacial Orthop* 2009;135:95–8.