Influence of lingual orthodontic therapy on microbial parameters and periodontal status in adults

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SUMMARY Insertion of fixed orthodontic appliances can induce an increase in oral biofilm and thereby cause inflammation of the periodontal tissues. The purpose of this study was to perform a longitudinal analysis of clinical and microbial parameters after insertion of lingual brackets.

Bleeding on probing (BOP), plaque index (PI), and pocket probing depth (PPD) were measured in 10 adults (8 females and 2 males, aged 29.0 ± 4.7 years) who received treatment with custom-made lingual appliances (Incognito/iBraces) before (T_0) and 3 months after beginning of treatment (T_1). No supportive dental prophylaxis was undertaken. In addition, a 16S rRNA-based polymerase chain reaction (PCR) method was used to detect *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) in the crevicular fluid. A Wilcoxon test was used to compare clinical parameters at the buccal (control) and lingual sites between T_0 and T_1.

At T_0, BOP was 12.4 ± 8.2 per cent, PPD 2.1 ± 0.3 mm, and PI 0.1 ± 0.2 at the buccal sites and at T_1 14.3 ± 8.1 per cent, 2.1 ± 0.2 mm and 0.1 ± 0.2, respectively. At the lingual sites, BOP was 22.2 ± 19.0 per cent, PPD 2.3 ± 0.3 mm, and PI 0.1 ± 0.2 at T_0 and at T_1 56.2 ± 31.6 per cent, 2.9 ± 0.3 mm, and 1.2 ± 1.1, respectively. Differences between T_0 and T_1 were significant for clinical parameters only at the lingual sites. Aa was found in five patients at baseline and in four at T_1, whereas Pg was found in one patient at T_0 and in two at T_1.

Insertion of fixed lingual appliances without supportive dental prophylaxis induced a worsening of clinical parameters restricted to the lingual sites, whereas the relative prevalence of Aa and Pg remained unchanged.

Introduction

In recent years, a higher demand for orthodontic treatment in adults has been observed (Scott *et al.*, 2007). For sociocultural reasons, aesthetics play an important role for adult patients when considering treatment. To provide invisible orthodontic treatment, fixed lingual bracket systems have been developed over the last 30 years (Fujita, 1979; Alexander *et al.*, 1982; Kurz *et al.*, 1982). These prefabricated lingual bracket systems were reported to cause problems such as speech dysfunction, restriction of mastication, and oral discomfort (Fujita, 1982; Fillion, 1997). Today, most problems associated with lingual appliances have been overcome as a result of customized brackets and computerized archwire fabrication (Fig. 1; Wiechmann, 2002, 2003; Stamm *et al.*, 2005).

However, orthodontic therapy using fixed buccal appliances induces iatrogenic side effects, e.g. an increase of plaque formation and caries (Øgaard, 1989; Hägg *et al.*, 2004). This increase of bacterial adhesion can be explained by the higher number of plaque-retentive sites and impaired mechanical plaque removal. These ecological changes induce a qualitative bacterial shift, with a higher prevalence of periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg; Paolantonio *et al.*, 1997; Lee *et al.*, 2005). Clinically,

this bacterial shift is associated with a higher incidence of side-effects in periodontal tissues. Orthodontic treatment affects the periodontium by facilitating plaque-associated gingivitis, contributing to gingival enlargement, an increase in pocket probing depth (PPD), and bleeding on probing (BOP; Ong and Wang, 2002). Research on the clinical side-effects of fixed orthodontic treatment has so far focused on buccal bracket systems. In the literature, there are only limited reports concerning the periodontal implications of lingual orthodontic treatment in adults (Hohoff *et al.*, 2003). To date, the influence of customized lingual treatment on microbial parameters has not been investigated. Therefore, the objective of the present study was to examine the influence of fixed lingual orthodontic treatment in relation to periodontal conditions in adult patients. Furthermore, a qualitative microbial analysis focused on Aa and Pg was performed.

Subjects and methods

This study was approved by the Ethics Committee of Hannover Medical School (No. 4347). The examination was performed with the understanding and written consent of each subject.

Figure 1 Bonded upper arch with customized lingual brackets at the start of treatment.

Power and sample sizes were calculated using nQuery Advisor 5.0 (Statistical Solutions, Saugus, Massachusetts, USA). Power calculation revealed that a sample size of 10 would have an 80 per cent power to detect a difference in means of 15 per cent, assuming that the standard deviation (SD) of the differences was 15 per cent. No dropouts were recorded during the study.

Ten patients, eight females and two males, aged between 23 and 36 years (mean 29.0 ± 4.7 years) who received orthodontic therapy with fixed lingual appliances (Incognito, T.O.P. Service, Bad Essen, Germany/iBraces, Lingualcare, Dallas, Texas, USA) in both arches were included in the study. Five patients were treated for correction of a Class II occlusion, four for anterior crowding in the upper and/or lower arch and one because of an excess of space in both arches. The orthodontic pre-treatment prevalence in the sample was 50 per cent. The patients were selected consecutively from those treated at the Department of Orthodontics, Hannover Medical School, by a total of six orthodontists with at least 5 years of professional experience. The patients fulfilled the following inclusion criteria: no systemic illness, non-smoker, not pregnant, no periodontitis (pocket depth lesser than or equal to 4 mm and no radiographic bone loss), absence of extensive dental restorations (e.g. crowns or bridges), other plaque stagnation areas (e.g. impacted teeth) or removable partial dentures, no pharmacological treatment or antibiotic therapy during or up to 4 months before the study, older than 18 years of age, and need for orthodontic treatment.

On the day of bracket placement, all patients received dental hygiene instruction but no professional tooth cleaning was performed during the period of the study. The patients were instructed to brush their teeth at least twice a day and to use interdental toothbrushes. Before bonding, all brackets were treated on the dental aspect of their surface with a silane (Rocatec; 3M Espe, Seefeld, Germany). Furthermore, the dental aspects of the brackets were coated with a thin layer of a resin (Phase II, Reliance Orthodontic Products, Itasca, Illinois, USA). The brackets were bonded indirectly (Maximum Cure, Reliance Orthodontic Products), using a tray system after enamel etching. After bonding, composite flash was removed. The archwires were ligated using elastic modules and stainless steel ligatures.

Before (T_0) and 3 months after bracket placement (T_1), a full periodontal examination including PPD, BOP, and plaque index (PI) was performed. PPD and BOP were obtained at six sites per tooth, whereas PI was determined for the buccal and oral sites separately (Silness and Löe, 1964; Lang *et al.*, 1990). All clinical examinations were carried out by the same clinician (AD) using a marked periodontal probe (WHO-DMS probe, Deppeler, Rolle, Switzerland). PPD was measured to the nearest millimetre on the scale. The following index teeth were included in the maxilla and mandible: first molar, first premolar, and central incisor. In extraction cases, the second premolar was used instead of the first premolar. Measurements were performed randomized in the first and third or second and fourth quadrants using permuted block randomization with block sizes of 10.

A 16S rRNA-based polymerase chain reaction (PCR) method was used to determine the prevalence of Aa and Pg in the crevicular fluid. At T_0 and T_1, samples of sulcus fluid were taken at the buccal and lingual sites of the index teeth using sterile paper points. After isolation of sampling sites from saliva with cotton rolls and gently drying with an air jet to prevent contamination, the sterile paper points were inserted for 15 seconds in the gingival sulcus. Pooled samples were stored in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) at −80°C.

Whole-genomic bacterial DNA was extracted using QIAmp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). Concentration was measured at 260 and 280 nm. Nucleotide sequences of the upstream and downstream primer for the detection of Aa were 5′-TAG CCC TGG TGC CCG AAG C-3′ and 5′-CAT CGC TGG TTG GTT ACC CTC TG-3′ (Kim *et al.*, 2005). For the detection of Pg, the nucleotide sequences were 5′-AGG CAG CTT GCC ATA CTG CG-3′ and 5′-ACT GTT AGC AAC TAC CGA TGT-3′ (Ashimoto *et al.*, 1996). Cross-reactions between species-specific primers were inhibited by using only one primer pair for any PCR process. Unspecific universal primers were used to detect bacterial contamination (5′-GAT TAG ATA CCC TGG TAG TCC AC-3′ and 5′-CCC GGG AAC GTA TTC ACC G-3′). Products of PCR were controlled with a DNA molecular size marker by gel electrophoresis. DNA templates of cultivated Aa and Pg were used as positive controls. Templates were validated by sequencing (Culture Collection, University of Gothenburg, Göteborg, Sweden).

Documentation and evaluation of the data were performed using the Statistical Package for Social Sciences Version 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Reproducibility of clinical measurements (PPD) was assessed by repeating measurements within a session and

calculated using the method of Bland and Altman (1986). The Kolmogorov–Smirnov test was applied to determine normal distribution. As the data were not normally distributed, a Wilcoxon test was used to compare clinical parameters on the control (buccal sites) and bonded surfaces (lingual sites) between T_0 and T_1. All tests were performed two tailed with a significance level of $P < 0.05$.

Results

With regard to the reproducibility of clinical measurements, the empirical SD for PPD was 0.01 ± 0.01 mm, indicating excellent reproducibility.

The results of the clinical examination are shown in Table 1 and in Figure 2a–c. BOP at the buccal sites was 12.4 ± 8.2 per cent at T_0 and 14.3 ± 8.1 per cent at T_1. PPD at the buccal sites remained mostly unchanged at 2.1 ± 0.3 mm at T_0 and 2.1 ± 0.2 mm at T_1, whereas PI was 0.1 ± 0.2 at T_0 and T_1. At the lingual sites, BOP was 22.2 ± 19.0 per cent at T_0 and 56.2 ± 31.6 per cent at T_1. PPD increased from 2.3 ± 0.3 mm at T_0 to 2.9 ± 0.3 mm at T_1 at the lingual sites, whereas PI was 0.1 ± 0.2 at T_0 and 1.2 ± 1.1 at T_1. The difference between T_0 and T_1 was statistically significant for all clinical parameters at the lingual sites ($P < 0.05$).

The results of the microbial analysis are summarized in Table 2. After PCR with universal primers, a distinct band was obtained after gel electrophoresis for all samples taken. Aa was found in five subjects at T_0 and in four at T_1. At T_0, Pg was present in one patient, and at T_1, two patients harboured Pg.

Discussion

Treatment with fixed buccal orthodontic appliances compromises oral health by increasing plaque formation that is associated with a worsening of clinical parameters such as BOP and PPD (Naranjo *et al.*, 2006). However, no investigations have been carried out in patients undergoing treatment with fixed custom-made lingual orthodontic appliances to determine whether they show similar clinical effects.

To avoid inter-observer differences in obtaining clinical parameters in the present study, all patients were examined
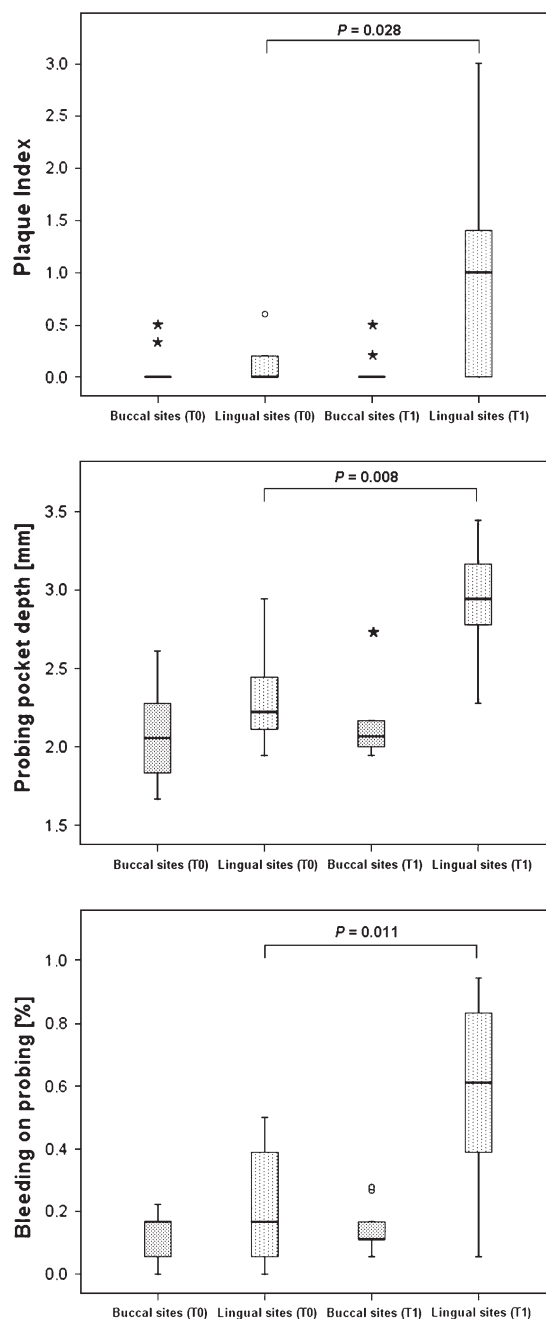

Figure 2 Plaque index (a), pocket probing depth (b), and bleeding on probing (c) at the buccal and lingual sites at baseline (T_0) and 3 months after treatment (T_1) with fixed lingual appliances.

Table 1 Clinical parameters at baseline and after 3 months of treatment with lingual appliances.

Clinical parameter		Baseline (T_0)	After 3 months (T_1)	*P* value
Bleeding on probing (%)	Buccal	12.4 ± 8.2	14.3 ± 8.1	0.349
	Lingual	22.2 ± 19.0	56.2 ± 31.6	0.011
Pocket probing depth (mm)	Buccal	2.1 ± 0.3	2.1 ± 0.2	0.339
	Lingual	2.3 ± 0.3	2.9 ± 0.3	0.008
Plaque index	Buccal	0.1 ± 0.2	0.1 ± 0.2	0.317
	Lingual	0.1 ± 0.2	1.2 ± 1.1	0.028

by the same clinician at T_0 and T_1. The investigation was performed over a specific period of 3 months to ensure a maximum increase of periodontal and microbial parameters (Ristic *et al.*, 2007). Clinical parameters were determined on six representative index teeth. The results of such an examination are largely comparable with those of a full periodontal examination (Beck *et al.*, 2006). Confounders such as preference to unilateral oral hygiene were taken into

Table 2 Prevalence of *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) in the patient pool.

Patient number	Before treatment (T_0)	After 3 months (T_1)
1	—	—
2	Aa	—
3	Aa, Pg	Aa, Pg
4	—	—
5	—	—
6	Aa	Aa
7	—	Aa
8	Aa	—
9	Aa	Aa, Pg
10	—	—

consideration by randomly selecting the first and third or second and fourth quadrant for clinical investigation.

A 16S rRNA-based PCR detection method was used to determine whether Aa and Pg were present at T_0 and T_1. This qualitative analysis was performed as a shift of periodontal parameters not only depends on the quantity of biofilm but also on the species in the biofilm (Nibali *et al.*, 2008). In previous studies, Aa was shown to be the predominant periodontal pathogen associated with fixed orthodontic treatment (Paolantonio *et al.*, 1996). Microbial species can be reliably identified by PCR. PCR also avoids time-consuming and error-prone cell culture (Lau *et al.*, 2004).

The clinical results of the present study showed a significant increase in lingual plaque formation after insertion of fixed lingual appliances. These results are in accordance with other clinical studies, which demonstrated higher plaque levels during fixed buccal treatment (Atack *et al.*, 1996; Paolantonio *et al.*, 1999). Higher amounts of biofilm are associated with an increase of PPD and BOP (Alexander, 1991). In the present study, this correlation was also found for lingual orthodontic appliances as PPD and BOP were significantly higher at lingual sites at T_1, whereas clinical parameters at the buccal sites remained unchanged over this period of time.

PCR was used to determine periodontal pathogens in adults. Although the patients were instructed and motivated by a dental hygienist before the start of treatment and none showed clinical signs of periodontitis, Aa was found in five and Pg in one patient at T_0. The positive findings of Aa and Pg at T_0 can be explained by the higher prevalence of periododontopathogenic microbiota in young adults. In a cross-sectional study, the natural occurrence of Aa and Pg in subgingival plaque was shown to be about 30 and 10 per cent, respectively, in adults under 30 years of age (Hamlet *et al.*, 2001). At T_1, without supportive dental prophylaxis, the occurrence of periodontal pathogens found in the present investigation was not significantly different. These results are in contrast to other findings (van Gastel *et al.*, 2007), which showed a bacterial shift towards an anaerobic microflora in the short term after insertion of fixed orthodontic appliances This contradiction might have been caused by the high level of Aa at baseline which could have camouflaged a bacterial shift.

Conclusions

The results of the present investigation show that orthodontic therapy in adults with fixed lingual appliances induced a worsening of clinical periodontal parameters restricted to lingual sites. A shift of periodontal microbiota towards an increased prevalence of the periodontal pathogens Aa and Pg was not observed. Consequently, plaque control seems to be important in lingual orthodontic therapy as well as in buccal treatment to maintain periodontal health.

In future studies with larger cohorts, the long-term effects of fixed custom-made lingual appliances on clinical and microbial parameters should be investigated. Furthermore, strategies to maintain periodontal health during fixed orthodontic treatment should be developed and implemented.

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