Original Article

Short-Term Influence of Lingual Orthodontic Therapy on Microbial Parameters and Periodontal Status

A Preliminary Study

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ABSTRACT

Objective: To perform a preliminary study of the short-term effect of fixed, customized lingual orthodontic appliances on periodontal and microbial parameters.

Materials and Methods: The sample comprised 20 subjects (6 males and 14 females) with a mean age of 22.3 years ± 8.6 years. Before (T_0) and 4 weeks after placement (T_1) of custom-made lingual appliances on the lower teeth only, plaque index (PI), probing pocket depth (PPD), and bleeding on probing (BOP) were measured. A 16S rRNA-based polymerase chain reaction (PCR) method was used to detect *Aggregatibacter actinomycetemcomitans* (*Aa*) and *Porphyromonas gingivalis* (*Pg*) in the crevicular fluid. To compare periodontal parameters on bonded lingual (testing) and unbonded palatal (control) and labial (control) sites between T_0 and T_1, the Wilcoxon test was applied.

Results: On the lingual aspects of bonded teeth, a significant increase of BOP (T_0: 23.4 ± 22.5%; T_1: 46.2 ± 23.5%; $P = .001$) and PI (T_0: 0.3 ± 0.3; T_1: 1.0 ± 0.7; $P = .001$) was observed, but no significant changes for PPD (T_0: 2.1 ± 0.4 mm; T_1: 2.2 ± 0.3 mm; $P = .286$) were found. On control sites, no significant changes were recorded for any periodontal parameter. *Aa* was found in 25% of the patients at baseline (5 subjects) and in 35% of the patients at T_1 (2 additional positive subjects), whereas *Pg* was found in 5% of the cohort at T_0 and at T_1 (same patient).

Conclusions: Even in the short term, insertion of fixed lingual appliances induced a worsening of periodontal parameters restricted to bonded lingual sites. (*Angle Orthod.* 2010;80:480–484.)

KEY WORDS: Lingual; Orthodontic; Microbial; Periodontal; Parameters; Pathogen

INTRODUCTION

"Invisible" orthodontic treatment can be provided by using fixed lingual bracket systems. In the past, prefabricated lingual bracket systems were reported to cause problems in clinical application due to difficult bracket and archwire insertion.[1,2] Furthermore, subjective impairments were reported by the patients, such as oral discomfort due to injury or irritation of the tongue, speech dysfunction as a result of a restricted functional space for the tongue, and restriction of mastication.[3] Due to the problems associated with prefabricated lingual appliances, customized brackets and computer-aided fabrication of individual archwires were implemented in lingual orthodontics.[4–6] Furthermore, the use of the indirect bonding technique simplified the insertion of lingual appliances.[7,8] As a result of the technical developments in recent years, most of the problems associated with lingual treatment have been overcome.

Due to increasing esthetic demands of young patients and, thanks to clinical simplification in using customized lingual appliances, the indication for lingual orthodontics today is no longer restricted to adults, but has been extended to adolescents, the major treatment cohort of orthodontists.[9]

After insertion of fixed labial orthodontic appliances, however, detrimental effects can be observed in the short and long term. As a result of ecological changes

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after bracket insertion, an increase in the amount, composition, metabolic activity, and pathogenicity of the oral microflora;[10–13] worsening of periodontal parameters; and an increase of incipient caries lesions can be observed.[14,15] Clinical studies have shown that a plaque-associated increase in periodontal probing depth (PPD) and bleeding on probing (BOP) can be observed during fixed orthodontic treatment with labial appliances.[16,17] Furthermore, labial bracket bonding affects the oral microflora by means of a higher prevalence of periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* (*Aa*) and *Porphyromonas gingivalis* (*Pg*).[11,18]

In the literature, there are numerous reports about the periodontal and microbial effects of labial orthodontic treatment, but only limited research has been performed considering this question with respect to fixed lingual appliances.[6,19] Therefore, the purpose of the present study was to perform an analysis of the short-term influence of fixed lingual appliances on the periodontal status and oral microflora. Especially the presence of *Aa* and *Pg* should be monitored during treatment with customized lingual brackets. The null hypothesis of this study was that insertion of fixed lingual appliances induces no differences of periodontal parameters on bonded sites after a period of 4 weeks.

MATERIALS 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.

The study group consisted of 20 patients (14 females and 6 males) aged between 12 and 36 years (mean, 22.3 $\pm$ 8.6 years). During the observation period, a fixed, customized lingual appliance (Incognito, TOP Service, Bad Essen, Germany and ibraces, Lingualcare, Dallas, Texas, USA) was inserted only on the mandibular teeth of all patients. Participants were selected consecutively from patients who were treated in the Department of Orthodontics of the Hannover Medical School by six orthodontists with at least 5 years of professional experience. The following exclusion criteria were defined: systemic illness, smoking, pregnancy, pocket depth $\geq$ 4 mm with radiographic bone loss, extensive dental restorations (eg, crowns or bridges), other plaque stagnation areas (eg, impacted teeth) or removable partial dentures, and pharmacological treatment or antibiotic therapy during or up to 4 months before the study.

During the study, no professional teeth cleaning was performed, but after baseline examination, all patients received accurate supragingival and subgingival ultrasonic scaling and dental hygiene instructions. Patients were informed to brush their teeth at least twice a day and to use interdental toothbrushes. According to the protocol of the manufacturer, the dental aspect of all brackets was treated with a silane (Rocatec; 3M Espe, Seefeld, Germany) before bonding; these surfaces were then coated with a thin layer of resin (Phase II, Reliance Orthodontic Products, Itasca, Ill). Bracket insertion was performed with the indirect bonding technique (Maximum Cure, Reliance), by using a tray system after enamel etching. After the bonding procedure, composite flash was removed. Elastic modules and stainless steel ligatures were used for the ligation of the initial NiTi archwire.

Periodontal Evaluation

A full periodontal examination including PPD, BOP, and plaque index (PI) was performed before (T_0) and 4 weeks after (T_1) bracket bonding in the mandible. PPD and BOP were obtained at six sites per tooth, whereas PI was determined for the labial and lingual sites separately.[20,21] Periodontal evaluation was carried out by the same trained clinician in all patients by using a marked periodontal probe (WHO-DMS probe Deppeler, Rolle, Switzerland). PPD was measured to the nearest millimeter 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. Randomized measurements were performed in the first (maxillary right) and third (mandibular left) or second (maxillary left) and fourth (mandibular right) quadrants using permuted block randomization with block sizes of 10.

Microbial Analysis

The prevalence of *Aa* and *Pg* in the crevicular fluid was detected by use of a 16S rRNA-based polymerase chain reaction (PCR) method. The PCR procedure was performed twice to ensure consistency of microbial data. Using sterile paper points, samples of sulcus fluid were taken at the labial and lingual sites on the index teeth at T_0 and T_1. Pooled samples were stored in Eppendorf tubes (Eppendorf AG, Hamburg, Germany) at -80°C.

Whole genomic bacterial DNA was extracted with a 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*,[22] *Pg*, and unspecific universal primers[23] are shown in Table 1. 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 (CCUG, Göteborg, Sweden).

Table 1. Species-Specific and Universal Primers for Polymerase Chain Reaction

Primer Pairs (5′–3′)
Aggregatibacter actinomycetemcomitans
5′-TAG CCC TGG TGC CCG AAG C-3′
5′-CAT CGC TGG TTG GTT ACC CTC TG-3′
Porphyromonas gingivalis
5′-AGG CAG CTT GCC ATA CTG CG-3′
5′-ACT GTT AGC AAC TAC CGA TGT-3′
Universal primers
5′-GAT TAG ATA CCC TGG TAG TCC AC-3′
5′-CCC GGG AAC GTA TTC ACC G-3′

Statistical Analysis

Power and sample sizes were calculated using nQuery Advisor 5.0 (Statistical Solutions, Saugas, Mass). Power calculation revealed that a sample size of 10 would have an 80% power to detect a difference in means of 15%, assuming that the standard deviation of the differences was 15%. Documentation and evaluation of the data were performed using the Statistical Package for the Social Sciences, Version 15.0 for Windows (SPSS Inc, Chicago, Ill). Reproducibility of clinical measurements (PPD) was assessed by repeating measurements within a session and calculated by applying the method of Bland and Altman.[24] The Kolmogorov-Smirnov test was applied to test for normal distribution. As data were not distributed normally, a Wilcoxon test was used to compare periodontal parameters on control (palatal/labial sites) and bonded surfaces (lingual sites) between T_0 and T_1. All tests were performed two-tailed with a significance level of $P < .05$.

RESULTS

No dropouts were recorded during the study. Results of the periodontal examination are shown in Table 2. At baseline, the mean values were similar for all periodontal parameters in testing and control sites.

Table 3. Prevalence of the Two Periodontal Pathogens, *Aggregatibacter Actinomycetemcomitans* (*Aa*) and *Porphyromonas Gingivalis* (*Pg*), Before and 4 Weeks After Bonding a Customized Lingual Appliance in the Mandible

Patient	Age	Before Bonding (T_0)	4 Weeks After Bonding (T_1)
1	32	–	–
2	15	–	–
3	33	–	–
4	17	–	–
5	24	–	*Aa*
6	29	*Aa*	*Aa*
7	33	*Aa*, *Pg*	*Aa*, *Pg*
8	13	–	–
9	16	–	–
10	28	–	–
11	13	–	*Aa*
12	23	*Aa*	*Aa*
13	13	–	–
14	12	–	–
15	36	*Aa*	*Aa*
16	30	–	–
17	33	*Aa*	*Aa*
18	17	–	–
19	12	–	–
20	17	–	–

Four weeks after insertion of the fixed, customized lingual appliance in the mandible, BOP and PI increased significantly at testing sites, whereas no changes of these parameters were found at control sites. PPD did not increase at testing or control sites during the observation period. Regarding the reproducibility of clinical measurements, the empirical standard deviation for PPD was 0.01 ± 0.01 mm, indicating excellent reproducibility.

After PCR with universal primers, a distinct band was obtained after gel electrophoresis for all samples taken. The microbiological data of specific primers are summarized in Table 3. At T_0, *Aa* was found in 25% of the patients (5 subjects), and at T_1, in 35% of the patients (2 additional subjects). *Pg* was present in 5% of the cohort at T_0 and T_1 (same patient).

Table 2. Periodontal Parameters at Baseline and 4 Weeks After Insertion of a Customized Lingual Appliance in the Mandible

			Baseline (T_0)	After 4 Weeks (T_1)	*P* Value
Plaque index	Maxilla	labial	0.2 ± 0.5	0.0 ± 0.1	0.223
		palatal	0.1 ± 0.1	0.1 ± 0.2	0.587
	Mandible	labial	0.2 ± 0.3	0.1 ± 0.2	0.329
		lingual	0.3 ± 0.3	1.0 ± 0.7	0.001
Bleeding on probing (%)	Maxilla	labial	19.9 ± 20.1	13.5 ± 13.6	0.184
		palatal	25.2 ± 19.2	22.2 ± 18.9	0.608
	Mandible	labial	18.1 ± 17.5	12.9 ± 16.7	0.101
		lingual	23.4 ± 22.5	46.2 ± 23.5	0.001
Probing pocket depth (mm)	Maxilla	labial	2.0 ± 0.3	2.0 ± 0.4	0.895
		palatal	2.1 ± 0.4	2.2 ± 0.5	0.184
	Mandible	labial	1.9 ± 0.4	1.9 ± 0.3	0.704
		lingual	2.1 ± 0.4	2.2 ± 0.3	0.286

DISCUSSION

In a recent study, it was shown that the intraoral location of biomaterials has an influence on in situ biofilm formation, indicating that initial biofilm thickness is reduced at palatal sites.[25] These findings might be due to tongue activity resulting in a self-cleaning mechanism of oral surfaces. Consequently, it might be assumed that the insertion of a fixed lingual appliance must not necessarily induce the periodontal and microbial effects that are well known from fixed labial orthodontic treatment.[26] Therefore, in our study, short-term effects of a lingual bracket system on clinical and microbial parameters were evaluated in a split-mouth setting.

The investigation was performed over the specific period of 4 weeks, because according to the manufacturer's protocol, insertion of the appliance in the maxilla is recommended after this period. A postponed insertion might lead to a bracket malpositioning, especially in adolescents, due to progression of tooth eruption. As a lingual control site was essential and a significant increase of periodontal and microbial parameters after this period was described for fixed labial treatment, a longer observation period was not used.[27]

The determination of periodontal parameters entails the risk of interobserver differences. Therefore, all patients were examined by the same clinician. Six representative index teeth were selected for the assessment of periodontal parameters. The results of such an examination are largely comparable with those of a full periodontal examination.[28] However, periodontal parameters were determined in a cohort of 20 patients. Therefore, the results of the present examination have to be considered as data of a preliminary study. Confounders, such as a tendency to unilateral oral hygiene, were taken into consideration by randomly selecting the first and third or second and fourth quadrant for clinical investigation.

The microbial analysis focused on *Aa* and *Pg* because these microorganisms are known to be the predominant pathogens of fixed labial orthodontic treatment.[29] Their determination was performed by use of a 16S rRNA-based PCR detection method, because this technique is less error prone and time consuming than bacterial cell culture.[30]

The null hypothesis was rejected because the results of periodontal examination showed a significant increase of PI and BOP on bonded lingual surfaces, whereas no changes for any parameter were found on palatal and labial control sites. Consequently, the lingual location of the appliance could not prevent the iatrogenic side effect of biofilm formation during fixed orthodontic therapy. Even the selection of gold alloy as a bracket material, which is known to have an influence on the composition, quantity, and vitality of the adherent biofilm,[31,32] could not prevent plaque formation and periodontal inflammation.

In contrast to PI and BOP, the PPD remained relatively constant over the observation period on testing sites. These results are in accordance with the results of another clinical study which also failed to find a significant increase in PPD on bonded labial sites after this period.[33]

The baseline prevalence of *Aa* and *Pg* in the present study can be described as the natural occurrence of these microbiota in this age cohort.[34] A slight bacterial shift was observed 4 weeks after lingual bracket bonding: the pretreatment prevalence of *Aa* was 25% and of *Pg*, 5%, and at T_1, 35% and 5%, respectively. However, long-term changes of microbial diversity after insertion of fixed lingual appliances were not evaluated in the context of this study. Results of the present examination largely agree with those of other studies, which showed a bonding site-specific colonization by *Aa* in labially treated orthodontic patients and a shift toward a higher concentration of periodontal pathogens.[33,35] Differing data between the cited studies and the results of the present investigation, especially baseline prevalence of periodontal pathogens, might be explained by the use of a bacterial cell culture method for the detection of periodontopathic microorganisms in contrast to the PCR method of our study. A further difference between the present investigation and the cited studies is the selection of patients, as the cohort of the present study entailed not only adults but also adolescents.

In this study, archwires were inserted by use of both elastic modules and stainless steel ligatures. In a recent study, the use of elastic modules for the ligation of orthodontic archwires was shown to harm gingival conditions and to favor the occurrence of periodontal pathogens.[36] So, to maintain periodontal health, we recommend an increased use of stainless steel ligatures in lingual orthodontic therapy as well. The results of this study stress the importance of dental prophylaxis even in lingual orthodontics to avoid periodontal side effects.

CONCLUSIONS

- The insertion of fixed lingual appliances induces an increase of plaque accumulation and gingival inflammation in the short term. These changes are restricted to bonding sites.
- A slight bacterial shift toward a periodontopathogenic microflora was observed for *Aa*, but not for *Pg*.

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