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ORIGINAL ARTICLE

Short-term effect of removal of fixed orthodontic appliances on gingival health and subgingival microbiota: A prospective cohort study

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Abstract

Objective. The aim of this prospective longitudinal study was to assess and compare the microbiological and clinical parameters of patients wearing a fixed orthodontic appliance, as opposed to 10 days after the bracket had been removed following treatment. ***Materials and methods.*** In total, 122 patients participated in this study; 61 of the subjects were assessed at baseline (wearing a fixed orthodontic appliance: T1) and 10 days after bracket removal (T2). The other 61 individuals had never worn an orthodontic appliance before and these subjects served as controls (CT). Subgingival plaque samples were assessed for bleeding on probing (GBI) and plaque index (VPI). PCR of 16s rDNA, followed by reverse species-specific hybridization for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* and *Treponema denticola* were performed. A descriptive analysis was conducted; chi-squared, Student's matched and unmatched *t*-tests, the point biserial correlation coefficient and the McNemar test were used to test for differences between groups ($p < 0.05$). ***Results.*** The GBI and VPI clinical parameters showed statistical differences ($p < 0.05$) between T1–T2, T1–CT and T2–CT. The prevalence of *T. denticola* had significantly decreased ($p = 0.039$) 10 days after appliance removal. At T2, a significant positive correlation was found between GBI and *A. actinomycetemcomitans* ($p < 0.01$) and between clinical parameters and *P. intermedia.* In patients without a fixed orthodontic appliance (T2 and CT), there was a significant positive correlation between *T. forsythia* and VPI. ***Conclusion.*** Local factors associated with the wearing of a fixed orthodontic appliance influence changes in subgingival plaque that leads to more inflammation and bleeding.

Key Words: *Periodontopathogens, orthodontics, microbiology, gingivitis*

Introduction

Fixed orthodontic appliances are bonded to the teeth surface, which seriously hampers proper oral hygiene and provides new possibilities for microorganisms to attach themselves. This causes biofilm formation and an increase in dental plaque. The complex biofilm structure provides nutrients and protection for periodontal pathogens. Fixed orthodontic appliances also retain food, which supplies nutrients—particularly when oral hygiene is poor—so increasing the possibilities of colonization. The composition of the subgingival microbiota may be affected, leading to colonization by micro-organisms associated with gingival inflammation and periodontal destruction [1].

Persistent gingivitis is frequently found during orthodontic treatment. In most cases gingival inflammation does not result in permanent periodontal loss [2–5], although some studies have reported significant clinical attachment loss during orthodontic treatment [6,7]. However, host and bacterial factors interact in regulating the progression of an initial gingivitis lesion to periodontitis [8]. Inflammatory gingival changes are suggested as being reversible after bracket placement [9,10] and after appliance removal [5,11].

Various studies have investigated periodontal and microbiological changes following orthodontic treatment. Three months after appliance removal, a significant reduction in the periodontopathogens found during orthodontic treatment was reported [12,13] as

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well as a significant decrease in the subgingival colony forming units (CFU aerobe/anaerobe) ratio [14]. Clinical parameters, such as plaque index, gingival index, periodontal probing depth and gingival crevicular fluid decreased significantly 6 months [15] and 3 months [14] after bracket removal. Normalization of the plaque and gingival indices have even been reported 1 month after removing a fixed orthodontic appliance [11,16]. However, although clinical changes in inflammation and gingival health are visible to professionals a few days after an appliance has been removed, there is no available information about microbiological changes that occur in this short-term, less than 2 weeks after removing an orthodontic appliance. The aim of our study, therefore, was to evaluate the effect of removing an orthodontic appliance on gingival inflammation and periodontopathogen carriage.

Materials and methods

Subjects and clinical procedures

A power analysis (G*Power 3) was performed before the study began; 61 subjects per group were required to demonstrate statistical significance with 80% power and an α error of 0.05. A total of 122 consecutive patients (aged 21.3 ± 5.6 [mean ± SD]) were, therefore, included in the study. All of the participants in the study were recent referrals to the Department of Orthodontics at the University of Seville, Spain. Subjects were enrolled according to the following criteria: (a) there was no visible alveolar bone loss on a panoramic X-ray; (b) no probing depth ≥ 4 mm at one or more sites; (c) healthy systemic condition; (d) not currently pregnant; (e) no periodontal treatment within the previous 6 months; (f) non-smokers; and (e) no history of diabetes. None of the participants had received antibiotic therapy within the previous 3 months and none were receiving antibiotic therapy during the experimental period. The present study was carried out with the full knowledge and written consent of each subject, in accordance with the ethical principles governing medical research and human subjects and with the approval of the Ethical Committee for Human Research at the University of Seville.

The patients were divided into two groups: 61 subjects were assessed at baseline (wearing a fixed orthodontic appliance due to be removed within 10 days; T1) and 10 days after the bracket had been removed (T2). The medium of the treatment of the sample was 31.6 (+4.3) months. The other group, 61 individuals, who had not previously worn an orthodontic appliance, formed the control group (CT).

The clinical parameters were taken at T1, T2 and CT, as previously described [17]. To provide a record of the state of the periodontium and the accumulation of dental plaque, the visible plaque index (VPI) [18] and gingival bleeding index (GBI) [18] were both recorded.

Subgingival samples were obtained by inserting sterile paper points to the bottom of the periodontal sulcus and kept in place for 20 s having previously eliminated the plaque and saliva. Experimental areas were isolated with sterile cotton balls to avoid contaminating the saliva. The subgingival plaque samples were taken from: (a) the distal aspect of the right maxillary second premolar; (b) the distal aspect of the right maxillary first premolar; (c) the distal aspect of the left mandibular first premolar; (d) the distal aspect of the right maxillary central incisor; and (e) the distal aspect of the right mandibular second premolar. If the tooth was not present or had been extracted previously, the mesial aspect of the adjacent mesial tooth was used. The five samples were pooled, so that the results were species-specific not site-specific, then sent to the laboratory within 24 h of sampling. The samples were processed upon arrival.

Polymerase chain reaction (PCR)

DNA was extracted using the DNeasy Spin Column kit (QIAGEN, Düsseldorf, Germany), in accordance with the manufacturer's instructions. PCR amplification was realized by multiplex PCR of 16S rDNA (microIDENT®, Hain Lifescience, Hehbren, Germany). A reaction volume of 50 ml, consisting of 5 ml of DNA extraction and 45 ml of reaction mixture containing 35 ml of primer/nucleotide mix (microDent®), 5 ml of ×10 PCR buffer, 5 ml of 25 mM MgCl2 and 1 U Taq polymerase (MBI Fermentas, Vilnius, Lithuania). PCR cycling comprised an initial denaturation step at 95°C for 5 min, 10 cycles at 95°C for 30 s and at 60°C for 2 min, 20 at 95°C for 10 s, at 55°C for 30 s and at 72°C for 30 s with a final extension step at 72°C for 10 min. Reverse hybridization was performed for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola* in accordance with microDent® kit instructions. Biotinylated amplicons were denatured and incubated at 45°C with strips coated with five species-specific probes. Streptavidin-alkaline phosphatase conjugate was added and positive hybridization products were visualized by adding a substrate.

Statistical analysis

The data obtained were analyzed using the SPSS 17.0 software for Windows (LEAD Technologies, Charlotte, NC). Univariate analysis of the results consisted of a descriptive analysis of the clinical and microbiological parameters. Frequency comparisons of periodontal pathogens between T1 and T2 were performed using the McNemar test. The chi-square test was used to compare the frequencies of

Table I. Clinical parameters (mean ± SD) for T1 patients (with brackets), T2 patients (10 days after removal of brackets) and control patients with no previous orthodontic treatment (CT, without brackets).

				T1 vs T2			T1 vs CT			T2 vs CT		
		CI			CI			CI				
	T1	T2	CT	*p*	Lower	Upper	*p*	Lower	Upper	*p*	Lower	Upper
VPI	65.95 ± 32.65	20.63 ± 22.65	48.03 ± 33.58	0.000*	38.77	51.87	0.003*	6.09	29.74	0.000*	37.62	17.17
GBI	63.89 ± 29.89	14.46 ± 21.32	27.44 ± 26.50	0.005*	41.78	57.07	0.000*	26.37	46.54	0.003*	21.56	4.37

*$p < 0.05$.
Aa, *A. actinomycetemcomitans*; Pg, *P. gingivalis*; Pi, *P. intermedia*; Tf, *T. forsythia*; Td, *T. denticola*.

periodontopathogens found in T1 vs CT and T2 vs CT sub-groups. Clinical differences between the experimental (T1 and T2) and CT groups were compared using bivariate analysis, 95% significance after verifying randomness, using the Student's *t*-test for independent samples (Wald-Wolfowitz runs test at $p > 0.05$ for all variables in both groups) and for normality (the Shapiro-Wilk test for normality at $p > 0.05$ for all variables in both groups). The paired sample *t*-test was used to compare clinical parameters between T1 and T2.

Binary logistic regression was used to determine associations between any of the bacterial complexes and the presence/absence of orthodontic fixed appliances. Differences were considered significant at $p < 0.05$.

Results

When compared to patients wearing an orthodontic appliance (T1), those not wearing one (T2 and CT) had significantly reduced values for clinical parameters VPI and GBI. Clinical parameters were significantly less in patients whose appliance had been removed (T2) compared to the control group with no previous orthodontic treatment (CT) (Table I).

The results of the periodontopathogen isolates found are shown as the percentage frequency of positive sites for each species at each time point sampled (Table II). Without an orthodontic appliance, there was a reduced, but not statistically significant ($p > 0.05$) prevalence of *T. forsythia* (T1 = 42.6; CT = 18.0), *A. actinomycetemcomitans* (T1 = 3.3%, CT = 1.6%), *T. denticola* (T1 = 29.5%; CT = 11.5%), *P. intermedia* (T1 = 16.4%; CT = 13.2) and *P. gingivalis* (T1 = 16.4; CT = 8.2) (Table II). Ten days after removal of the fixed orthodontic appliance (T2), there was a significant decrease ($p = 0.039$) in *T. denticola* compared to 10 days before the appliance was removed (T1).

The results of looking for the presence of none or one, two or three of the pathogens that form the red complex [19] in any of the three groups revealed that young adults wearing orthodontic appliances (T1) were associated with the presence of two ($p = 0.043$) or three ($p = 0.047$) pathogens from the so-called red complex when compared to the control group (CT). Moreover, the orthodontically treated group after the orthodontic appliance had been removed was associated with the presence of two of the red complex pathogens, compared to the control ($p = 0.007$). Differences of bacterial complexes between subjects wearing orthodontic appliance (T1) and post-treatment subjects (T2) were not found to be statistically significant ($p > 0.05$). Nevertheless, results extracted from the distribution graphics show that those subjects

Table II. Prevalence of periodontal pathogens in T1 patients (with brackets), in T2 patients (10 days after removal of brackets) and control patients with no orthodontic treatment (CT, without brackets).

	Prevalence of periodontal pathogens (%)					
Pathogen	T1 (n = 61)	T2 (n = 61)	CT (n = 61)	T1 vs T2[a]	T1 vs CT[b]	T2 vs CT[b]
Aa	3.3	1.6	1.6	1	0.853	0.896
Pg	16.4	18.0	8.2	1	0.137	0.182
Pi	16.4	14.8	13.1	1	0.179	0.207
Tf	42.6	41.0	18.0	1	0.834	0.089
Td	29.5	16.4	11.5	0.039*	0.348	0.213

Aa, *A. actinomycetemcomitans*; Pg, *P. gingivalis*; Pi, *P. intermedia*; Tf, *T. forsythia*; Td, *T. denticola*.
[a]McNemar test.
[b]Chi-square test.
*McNemar test indicated significant difference ($p < 0.05$) in frequency of *T. denticola* with respect to the wearing of brackets.

undergoing treatment presented three pathogens from the red complex, while there was no presence of this association in the control or post-treatment group (Figure 1).

Placing bands, therefore, promoted increased growth of the various marker organisms in some of our subjects. Banding promoted the occurrence of members of the 'red complex' in some of the subjects; only ~ 10% of subjects with banding, however, presented all three members of the red complex and only when bands were present. The *A. actinomycetemcomitans* and *P. intermedia* organisms occurred in some subjects, irrespective of whether bands were in place or not. Approximately half the subjects with brackets and 60% of the control subjects never presented any marker organisms at any time during the study.

Discussion

This study focused on the short-term effect —just 10 days—of removing a fixed orthodontic appliance, since microbial and clinical periodontal data in this respect are lacking. Different authors have investigated changes in clinical and periodontal pathogens at 6 months [15], 3 months [12–14] or 1 month [16] following appliance removal and all coincide in describing a decrease in the prevalence of putative periodontopathogens and an improvement in gingival health after orthodontic treatment. When attachment loss was assessed [14,16], there was less probing depth found once the appliance was no longer there. In fact, most longitudinal studies [2,3,5] concentrating on clinical effects during treatment concluded that attachment loss in individuals undergoing fixed orthodontic treatment was transient and that no permanent damage could be observed [20]. However, the most obvious clinical feature a few days after removing an orthodontic appliance is the improvement to gingival inflammation. Despite this, no studies to date have focused on changes in gingival inflammation and subgingival microbiota a few days after orthodontic treatment has finished.

In our study, there was a decreased prevalence of four of the five putative periodontopathogens studied 10 days after removing a fixed orthodontic appliance: *A. actynomicetemcomitans* (T1 = 3.3%; T2 = 1.6%; $p > 0.05$), *P. intermedia* (T1 = 16.4%; T2 = 14.8%; $p > 0.05$), *T. forsythus* (T1 = 42.6%; T2 = 41.0%; $p > 0.05$) and *T. denticola* (T1 = 29.5%; T2 = 16.4%; $p < 0.05$). This phenomenon has been demonstrated in other studies where a non-significant decrease in the putative periodontopathogens was found 3 months after appliance removal [12,13]. Surprisingly, in our study, there was an increase in *P. gingivalis* (T1 = 16.4%; T2 = 18.0%; $p > 0.05$) and our result agrees with those of longitudinal studies carried out during and after orthodontic treatment [13]. In short, 10 days after removing an orthodontic appliance, small reductions were observed in the prevalence of four putative periodontopathogens, accompanied by a statistically significant improvement in the plaque and gingival indexes. *T. denticola* was found to be the only pathogen that decreased in a significant manner following appliance removal. The role and specific implication of this oral spirochete in periodontitis have already been demonstrated [20,21], as changes in the subgingival ecology of diseased sites have been strongly associated with *T. denticola* [22,23] and it is frequently isolated in severely diseased sites in periodontitis patients [24,25]. The molecular signaling mechanism of this major contributor to tissue destruction in periodontal disease [23] was recently explained [26] as the production of components able to mediate adherence to mucosal surfaces, enable penetration into epithelial cells and affect host systems through specific cleavage of cell surface receptors [22]. However, it not only contributes to tissue destruction and alveolar bone resorption, but inhibits host defense mechanisms and elicits inflammation in gingival tissues [22]. So, the presence of *T. denticola* in healthy periodontal sites is also associated with an increased susceptibility to gingival inflammation [27,28]. In orthodontic patients, *T. denticola* was also significantly more prevalent in gingivitis lesions where there was no alveolar bone loss, than in the gingivitis lesions of subjects who did not wear orthodontic appliances [29]. This finding could indicate that an orthodontic appliance increases the prevalence of this periodontopathogen, supragingival plaque and the gingivitis that occurs in patients who wear appliances. Ten days after removing brackets, there was a statistically significant reduction in plaque levels, gingival inflammation and the prevalence of *T. denticola*.

The findings of the present study are very similar to others [12–15] that found more or less the same overall results: when bands/brackets are placed, periodontitis-associated bacteria increase in numbers and, when the bands/brackets are removed, there is a general decrease towards the occurrence of bacteria in normal individuals. All these studies examined adolescent populations. The present paper is unique in looking at a slightly older population of individuals—young adults—receiving orthodontic treatment, which is closer to the age group when orthodontic treatment has the potential for becoming associated with an increased risk of periodontitis in some adults [30]. The aim of the present paper was to look for clustering of marker bacteria occurring in individuals, as described above for the red complex distributions (Figure 1). The 'red complex' was only present as an actual complex of three organisms in patients with brackets present and only in 11% of such patients. Approximately half the subjects with brackets and 60% of the control subjects never presented any marker organisms during the study.

The association between orthodontic treatment, worsening oral hygiene and gingivitis has largely

Figure 1. Distribution of the different microbiological complexes within the three groups [T1, T2, CT]. All three members of the 'red complex' were present together in subjects only when brackets were present and only in seven of the 61 subjects with brackets (11%). Approximately half the subjects with brackets never had any marker organisms present during the study. Approximately 70% of the control subjects never had any members of the 'red complex' present, while 60% of control subjects never had any marker organisms present during the study. RC0 = none of the pathogens of the 'red complex'; RC1 = one of the pathogens of the 'red complex'; RC2 = two of the pathogens of the 'red complex'; RC3 = all three of the pathogens of the 'red complex' are present.

been demonstrated [31–34]. The presence of a fixed appliance tends to be a factor making it easier for plaque to accumulate and be retained, while the changing supragingival and subgingival environment can increase plaque around the teeth; gingival health did not worsen as a result of orthodontic treatment, however, if good oral hygiene was maintained [3]. GBI and VPI results were found lower 10 days after removing a fixed orthodontic appliance than in control patients. According to these data we hypothesized that patients that just have finished orthodontic treatment are more sensible to oral hygiene and insists more in techniques of oral hygiene than control patients. In this study, we used the GBI to assess gingival inflammation and the VPI to assess oral hygiene. Our results showed a weak association between *P. intermedia* and GBI (0.391, $p < 0.01$) and VPI (0.310, $p < 0.05$) after removing the appliance and between the plaque index (VPI) and *T. forsythus* where an orthodontic appliance had never been worn (T2 = 0.273, $p < 0.05$; CT = 0.285, $p < 0.05$). In this study, *A. actinomycetemcomitans* correlated positively ($p < 0.01$) with GBI after appliance removal, but not in subjects who were wearing orthodontic appliances (T1). An over-abundance of other harmful species could conceal the real pathogenicity of *A. actinomycetemcomitans*. This finding appears to be highly consistent with those of other other studies [20,29]. There was a decrease in the prevalence of *A. actinomycetemcomitans* between T1 and T2. *A. actinomycetemcomitans* (1.6%) was also shown to be prevalent in control subjects (CT), which is in agreement with other studies which have shown this periodontal pathogen in healthy subjects and in sites unaffected by gingivitis [20,35,36]. It would be interesting for future studies to review the future periodontal status of the subjects of this or similar research. It would also be of great interest to discover whether there is any useful predictive information available about the finding of specific marker bacteria in those subjects associated with periodontal stability/periodontal disease.

In conclusion, 10 days after removing a fixed orthodontic appliance there was a significant reduction in gingival inflammation, the plaque index and in the prevalence of *T. denticola*. Our results suggest that the presence of *A. actinomycetemcomitans* is associated with short-term bleeding following orthodontic treatment.

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