

The monitoring of gingival crevicular fluid volume during orthodontic treatment: a longitudinal randomized split-mouth study

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SUMMARY This randomized split-mouth study was aimed at evaluating whether an orthodontic appliance *per se* or orthodontic tooth movement can induce detectable changes in gingival crevicular fluid (GCF) volume, and thus whether GCF volume is a predictable biomarker for tissue remodelling incident to orthodontic tooth movement. **Materials and Methods:** Sixteen healthy orthodontic patients (7 females and 9 males; mean age, 17.7 years; range, 13–27 years) with the need for extraction of the first upper premolars were enrolled. One randomly chosen maxillary canine was subjected to a distalizing force by a 0.017 × 0.025 inch titanium-molybdenum alloy archwire and considered as the test tooth (TT). The contralateral canine, which was not subjected to any force but was included in an orthodontic appliance, was used as a control (CT). GCF sampling was performed at both mesial and distal sites of the CTs and TTs at baseline, immediately before applying the orthodontic appliance, and after 1 hour, 24 hours, and 7, 14, and 21 days. A Periotron was used to measure the GCF volume.

A modest but significant increase in the GCF volume over time was seen in both the CTs (mesial sites) and the TTs (both mesial and distal sites) with no differences between the experimental teeth.

Subclinical tissue inflammation consequent to the placement of the orthodontic appliance might be responsible for these GCF volume changes. The GCF volume does not appear to be a reliable biomarker for tissue remodelling during orthodontic treatment.

Introduction

Gingival crevicular fluid (GCF) is a complex mixture of substances released within the crevicular sulcus that are derived from serum, host cells, and oral bacteria (Uitto, 2003). The GCF is a transudate of interstitial tissues produced by an osmotic gradient (Pashley, 1976). However, during periodontal inflammation, the main mechanism of GCF formation becomes exudative (Alfano, 1974).

In recent years, many cell mediators and enzymes from the GCF have been investigated for their predictive value in monitoring tissue loss in periodontitis (Lamster, 1992; McCulloch, 1994). Interestingly, previous investigations have showed that the amount of GCF increases during gingivitis (Griffiths *et al.*, 1992) and periodontitis (Egelberg, 1966). Under normal conditions, about 3 l/hour of fluid is released into the crevicular sulcus, while during periodontitis, up to 44 l/hour of release has been reported (Goodson, 2003). Therefore, the monitoring of the GCF volume has been proposed as a better indicator of gingival inflammation than standard clinical procedures (Griffiths *et al.*, 1992).

Considering that tissue remodelling incident to orthodontic tooth movement is triggered by an inflammatory process in which one of the first events is an increase in vascular permeability (Krishnan and Davidovitch, 2006), it has been hypothesized that the amount of GCF production might reflect these tissue changes. However, when analysing the effects of orthodontic tooth movement on the amount of GCF, contrasting results have been reported. Indeed, both increased (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994; Baldwin *et al.*, 1999; Tuncer *et al.*, 2005; Basaran *et al.*, 2006a,b) and unchanged (Uematsu *et al.*, 1996; Perinetti *et al.*, 2002; Apajalahti *et al.*, 2003; Serra *et al.*, 2003; Sugiyama *et al.*, 2003; Yamaguchi *et al.*, 2006; Dilsiz *et al.*, 2010) amounts of GCF production have been reported in human studies. However, these previous investigations were based on very short monitoring following the application of the forces, i.e. 1 week (Uematsu *et al.*, 1996; Apajalahti *et al.*, 2003; Sugiyama *et al.*, 2003; Tuncer *et al.*, 2005; Yamaguchi *et al.*, 2006; Dilsiz *et al.*, 2010), and they were also cross-sectional (Serra *et al.*, 2003), did not use a split-mouth design (Last *et al.*, 1988), did not

investigate any differences between tension and compression sites (Basaran *et al.*, 2006a,b), or had no control group (Pender *et al.*, 1994; Baldwin *et al.*, 1999).

The present randomized split-mouth study aimed at evaluating whether an orthodontic appliance *per se* or orthodontic tooth movement can induce detectable changes in GCF volume, with examination of the proposal that GCF volume can be used as a predictable biomarker for tissue remodelling incident to orthodontic tooth movement.

Materials and methods

Study population and experimental design

Sixteen non-smoking orthodontic patients, 7 females and 9 males (mean age, 17.7 years; range, 13–27 years), were enrolled in the study. The following inclusion criteria were followed: 1. need for fixed appliance therapy involving extraction of the first upper premolars and distal retraction of the maxillary canines, 2. good general health, 3. no use of anti-inflammatory drugs in the month preceding the beginning of the study, 4. probing depth values not exceeding 3 mm in the whole dentition, and 5. full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) ≤ 30 and ≤ 10 per cent, respectively, recorded as the percentage of teeth with the presence of supragingival plaque (PL) or bleeding on probing (BOP; Perinetti *et al.*, 2004). The FMPS and FMBS were recorded in a preliminary examination, which took place 7 days before the beginning of the study and no less than 1 month after the first premolar extraction.

During the month preceding the preliminary examination, all the subjects received repeated oral hygiene instructions about the correct use of a toothbrush, dental floss, and interdental brush. Moreover, the study subjects were not allowed to take any anti-inflammatory drugs during the study that might affect the results (Perinetti *et al.*, 2002). At the beginning of the study (baseline), an orthodontic appliance was mounted. In particular, one randomly chosen maxillary canine was subjected to a distalizing force and considered as the test tooth (TT); the contralateral canine, which was not subjected to any force but was included in an orthodontic appliance, was used as a control (CT). The samplings of GCF were performed at both mesial and distal sites of the CTs and TTs, at baseline, immediately before the mounting of the orthodontic appliance, and after 1 hour, 24 hours, and 7, 14, and 21 days. The clinical parameters were collected at baseline and at 7, 14, and 21 days. At each time point, including the preliminary visit, repeated oral hygiene instructions relating to the correct use of a toothbrush, dental floss, and an interdental brush were given to the subjects to inform them how to perform effective tooth cleaning in the presence of the orthodontic appliance. Moreover, a kit containing toothpaste, toothbrush, and chlorhexidine gluconate mouth rinse was also given to each of the patients to help with their home care dental hygiene procedures.

Informed consent was obtained from the patients, and the parents of patients under 18 years of age, prior to the start of the study. The protocol was reviewed and approved by the Ethical Committee of the State University of Rio de Janeiro, Brazil.

Orthodontic appliance

The orthodontic appliance is shown in Figure 1. On the upper arch, and on both the right and the left sides, bands were mounted on the first molars, while brackets were bonded onto the second premolars and canines (Morelli, Sorocaba, Brazil; Iwasaki *et al.*, 2000). A 0.019×0.025 inch stainless steel passive archwire (Morelli) segment was inserted into the tube of the molar band and tied to the bracket on the second premolar. Moreover, a 0.017×0.025 inch titanium-molybdenum alloy archwire (Morelli) segment was inserted into the auxiliary tube of the molar bands, bent so as to have a mesial vertical loop, and tied to the bracket on the canine. Only the TT was subjected to the distal force, which was provided by a NiTi closed coil spring (Morelli; Figure 1 top); this was not applied on the contralateral CT (Figure 1, bottom). The NiTi closed coil spring was mounted between the vertical loop, with a crimpable ball hook, and to the molar hook, and a force gauge (Dentaurum, Ispringen, Germany) was used to set the force exerted by the coil spring to 150 g. To further anchor the first molars, a transpalatal bar was also mounted. The entire orthodontic appliance was placed in a single clinical session by the same orthodontist. No orthodontic appliance was placed on the mandibular arch during this visit or throughout the study period.



Figure 1 The orthodontic appliance on the test tooth (top) and control (bottom). See text for details.

Clinical monitoring and GCF collection and measurement

Periodontal examinations were carried out at six sites per tooth (mesio-, mid-, and disto-buccal and mesio-, mid-, and disto-lingual/palatal sites). The clinical examinations consisted of recording the PL, assessed by visual criteria, and assessing BOP within 15 seconds after probing with a 20 g controlled force probe. A tooth was scored as positive (PL+ or BOP+) if any of the corresponding sites showed visible plaque or bleeding. Clinical data were always collected by the same operator. Contamination of the GCF samples was minimized by recording the PL before carefully cleaning the tooth with cotton pellets, collecting the GCF from the isolated area, and then recording the BOP (Perinetti *et al.*, 2002). Moreover, no brushing procedures were allowed immediately before the GCF sampling to avoid mechanical injuries to the periodontal tissues.

Each crevicular site included in the study was isolated with cotton rolls. Before GCF collection, a gentle air stream was directed towards the tooth surface for 5 seconds to dry the area. GCF was collected in every site by the use of a periopaper strip (IDE Interstate, Amityville, New York, USA) inserted into the gingival crevice and left *in situ* for 30 seconds. Immediately after collection, the strips were positioned on the gingival fluid measurer Periotron 8000 (IDE Interstate), which determined the exact GCF volume in each paper strip. The Periotron was previously calibrated using human serum and was used according to the manufacturer's instructions.

Data treatment

The Statistical Package for Social Sciences Software 13.0 (SPSS Inc., Chicago, Illinois, USA) was used for the data analysis. Parametric methods were used after testing the normality of the data, using a Shapiro–Wilk test and Q–Q normality plots; the equality of variance among the datasets was also tested using a Levene's test and Q–Q normality plots of the residuals. Otherwise, non-parametric methods were used.

The significance of the differences in the %PL+ and %BOP+ over time for the whole study population was evaluated using a Friedman test. The significance of the differences in the number of TTs and CTs PL+ and BOP+ over time was assessed by a Cochran test, while the differences between the experimental teeth within each time point were assessed by a McNemar test.

A repeated measure three-way analysis of variance (ANOVA) was performed to assess the differences in the GCF volumes. The three factors in the ANOVA were treatment (experimental group), site, and time. A paired sample *t*-test was used when appropriate for pairwise comparisons. A *P* value less than 0.05 was used for rejection of the null hypothesis, and appropriate Bonferroni corrections were applied to adjust the *P* values in the pairwise comparisons.

Results

Detectable distal movement of different magnitudes was seen with all the TTs, while the CTs did not show any clinically detectable displacement (not shown). The clinical data regarding the %PL+ and %BOP+ are shown in Table 1. The mean %PL+ ranged from 18.9 to 27.3 and the mean %BOP+ from 1.3 to 3.9. Both these parameters showed small reductions over time, although these were not statistically significant. The overall number of TTs and CTs positive for PL was less than 1 per time point, while only in a single case was a BOP+ score assigned to a CT (baseline).

The GCF volume data are shown in Table 2. The mean values ranged from 0.40 l (TTs mesial sites, 7 days) to 0.69 l (TTs distal sites, 14 days). Generally, a slight increase in GCF volume was seen over time and on the distal sites, in both the experimental groups. At the three-way ANOVA, both the site and the time factors yielded statistically significant differences ($F_{1;15} = 18.87$, $P = 0.001$ and $F_{1;15} = 5.72$, $P = 0.000$, respectively). In contrast, the variance due to the treatment did not reach statistical significance ($F_{1;15} = 0.41$, $P = 0.530$). Among all the two-way and three-way interactions, none reached statistical significance. In more detail, the differences over time within each experimental group and site were statistically significant in the TTs, at both sites, and the CTs, at the mesial sites. At the pairwise comparisons, no significant differences were seen between the sites within each experimental group and time; only in the TTs, the distal sites showed significantly greater GCF volumes recorded at 14 days as compared to the corresponding baseline values.

Discussion

The present randomized split-mouth study shows that tissue remodelling consequent to orthodontic tooth movement does not produce any clinically relevant increase in GCF volume over the first month of treatment. The use of biomarkers in orthodontics is advocated for non-invasive monitoring of tissue remodelling during orthodontic

Table 1 Clinical parameter changes in the study population over time ($n = 16$).

Analysis	PL+ (% of positive sites)		BOP+ (% of positive sites)	
	Mean \pm SD	Median	Mean \pm SD	Median
Time				
Baseline	27.3 \pm 17.7	28.7	3.9 \pm 5.8	0.0
7 days	18.9 \pm 17.9	19.3	2.2 \pm 3.4	0.0
14 days	19.1 \pm 16.2	20.3	2.2 \pm 8.7	0.0
21 days	22.6 \pm 19.7	16.7	1.3 \pm 3.4	0.0
Diff.	NS		NS	

Diff., significance of the differences over time; NS, no statistically significant difference.

Table 2 GCF volume in the different experimental groups according to sites and over time ($n = 16$).

Time	GCF volume (l)					
	TTs		Diff.	CTs		Diff.
	Mesial	Distal		Mesial	Distal	
Baseline	0.44 ± 0.18	0.49 ± 0.17	NS	0.51 ± 0.18	0.54 ± 0.19	NS
1 h	0.51 ± 0.31	0.50 ± 0.16	NS	0.47 ± 0.16	0.61 ± 0.22	NS
24 h	0.51 ± 0.18	0.66 ± 0.21	NS	0.52 ± 0.11	0.59 ± 0.17	NS
7 days	0.40 ± 0.15	0.54 ± 0.19	NS	0.42 ± 0.16	0.52 ± 0.17	NS
14 days	0.55 ± 0.20	0.69 ± 0.20*	NS	0.60 ± 0.25	0.66 ± 0.16	NS
21 days	0.57 ± 0.16	0.61 ± 0.16	NS	0.55 ± 0.15	0.64 ± 0.22	NS
Diff.	$P = 0.040$; S	$P = 0.002$; S		$P = 0.023$; S	$P = 0.094$; NS	

Data are expressed as mean ± SD. Diff., significance of the difference between the sites within each experimental group and time point or over time within each experimental group and site. Asterisk indicates significantly different as compared to the corresponding baseline score at pairwise comparisons. S, statistically significant difference; NS, no statistically significant difference. All the differences between the experimental groups were not statistically significant.

treatment on an individual and site-specific basis. This is particularly true in the setting of the optimum force magnitude, which is hardly determined *in vivo* (Ren *et al.*, 2003). Previous studies have reported significant changes in the levels of several GCF constituents, including markers of inflammation (Uematsu *et al.*, 1996; Tuncer *et al.*, 2005; Basaran *et al.*, 2006a,b; Yamaguchi *et al.*, 2006), bone remodelling (Last *et al.*, 1988; Samuels *et al.*, 1993; Perinetti *et al.*, 2002), and tissue necrosis (Serra *et al.*, 2003). However, the monitoring of these markers requires a dedicated set-up for their quantification. On the contrary, the GCF volume can be determined easily and cheaply using a Periotron, which has been reported to have acceptable error measurements for samples greater than 0.2 l (Chapple *et al.*, 1999).

In the present study, optimal clinical conditions were seen in all the patients, due to the repeated oral hygiene instructions given and their regimen of chlorhexidine-based mouthwashes. The overall percentages of teeth PL+ and BOP+ were very low in the whole mouth throughout the study (Table 1). Moreover, the numbers of TTs and CTs positive for PL or BOP were not relevant. The slight, although not significant, improvement of these PL+ and BOP+ parameters could be attributed to an improved capability of the subjects to keep their oral hygiene during the study terms and their awareness of being included in a clinical trial.

The GCF volumes recorded were similar between the TTs and CTs at all the time points and between the mesial and distal sites within each experimental tooth (Table 2). However, a significant increase in GCF volume was seen over time in both the CTs (mesial sites) and the TTs (both mesial and distal sites). Considering the lack of orthodontic force on the CTs, this increase in GCF volume seen for these teeth can only be ascribed to the placement of the orthodontic appliance, responsible for a subclinical inflammation (see below). In this regard, even if minimal

forces might have been exerted from the passive archwires to the CTs, these teeth did not undergo to detectable displacement, with consequent not significant effects on the GCF volume. Therefore, the lack of significant differences between the TTs and CTs indicates that the tooth movement of the TTs did not alter the GCF volume. The greatest GCF volume changes were seen for the distal sites of the TTs at 14 days, as compared to the corresponding baseline values, with a mean increase of less than 50 per cent (by 0.69–0.49 l, respectively, Table 2).

To correctly compare the present data with previous findings, an important concept for consideration is the sampling procedure used to collect the GCF (Griffiths, 2003). The use of paper strips allows the collecting of the resting GCF inside the crevice, which is referred as to the GCF volume. In contrast, the use of capillary tubing kept inside the crevice for several minutes is useful for the measurement of the rate of GCF flow, which is a different entity. Previous evidence has shown differential behaviours of the GCF volumes and flow rates under gingival inflammation (Persson and Page, 1990; Griffiths *et al.*, 1992). In particular, only the GCF flow rate appears to increase during clinically detectable gingival inflammation, while the GCF volume appears to be less responsive to the actual clinical condition (Persson and Page, 1990; Griffiths *et al.*, 1992). Interestingly, in most of the previous studies that used paper strips to collect the GCF, there were no reports of significant changes in terms of GCF volume during orthodontic tooth movement (Uematsu *et al.*, 1996; Perinetti *et al.*, 2002; Apajalahti *et al.*, 2003; Serra *et al.*, 2003; Sugiyama *et al.*, 2003; Yamaguchi *et al.*, 2006; Dilsiz *et al.*, 2010); however, all the studies that used capillary tubing reported particular increases in GCF flow rate (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994). Unfortunately, these studies (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994) did not discriminate between tension and compression sites. Therefore, the present data would be

consistent with the existence of a reservoir of GCF inside the crevicular sulcus that would be poorly sensitive to tissue remodelling that is incident to orthodontic tooth movement.

Although the clinical parameters did not show significant differences over time or site, and although they were optimal throughout the study, subclinical inflammation in the experimental sites cannot be excluded. The composition of the subgingival plaque after the placement of an orthodontic appliance might have been responsible for undetectable inflammation (Perinetti *et al.*, 2004). These effects might eventually overcome any increases in GCF volume arising from the tissue remodelling that is incident to the orthodontic tooth movement, as previously suggested (Perinetti *et al.*, 2002). However, even though significant, these increases in GCF volume were small (Table 2), and they are probably without clinical meaning when the orthodontic appliance placement is concomitant with optimal clinical conditions. Further studies evaluating the rate of GCF flow in relation to orthodontic tooth movement in tension and compression sites are warranted.

Conclusion

The GCF volume is not a reliable biomarker for tissue remodelling during orthodontic treatment.

Acknowledgements

The authors are deeply grateful to Dr Tiziano Baccetti (University of Florence, Florence, Italy) for useful discussions and to Dr Christopher Paul Berrie (Telethon Institute for Genetics and Medicine, Naples, Italy) for critical appraisal of the manuscript.

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