

Matrix metalloproteinases -1, -2, -3, -7, -8, -12, and -13 in gingival crevicular fluid during orthodontic tooth movement: a longitudinal randomized split-mouth study

Cristiane Canavarro*, Ricardo PalmierTeles** and Jonas Capelli Júnior*

*Department of Orthodontics, School of Dentistry, State University of Rio de Janeiro, Brazil, **Department of Periodontology, The Forsyth Institute, Boston, MA, USA

Correspondence to: Cristiane Canavarro, Department of Orthodontics, State University of Rio de Janeiro, Boulevard vinte e oito de setembro, 157/230, Vila Isabel, Rio de Janeiro, RJ 20551-030, Brazil. E-mail: cristianecanavarro@gmail.com

SUMMARY This randomized split-mouth study aimed to examine the levels of matrix metalloproteinases (MMPs) -1, -2, -3, -7, -8, -12, and -13 in the gingival crevicular fluid (GCF) at different time points during orthodontic tooth movement. A total of 16 healthy orthodontic subjects (7 females, 9 males; mean age, 17.7 years) who needed their first upper premolars extracted were enrolled. One randomly chosen maxillary canine was subjected to a distalizing force and was considered to be the test side. The contralateral canine, which was not subjected to any force but was included in the orthodontic appliance, was used as a control side. GCF sampling was performed at both the mesial (tension) and distal (pressure) test and control sites at baseline, immediately before applying the orthodontic appliance, and after 1 and 24 hours and 7, 14, and 21 days. A multiplexed bead immunoassay was used to analyse the GCF samples. The mean levels of the MMP-1, -2, -3, -7, -8, -12, and -13 were not significantly different between the test and control groups in each time showed. The comparisons between the tension and pressure sites were also not significantly different at each individual time. A few variations focused on MMP-1 and -3, but the expression of MMP-8 was higher than that of the other MMPs. MMPs are released in sufficient quantities such that tooth movement occurs but with no significant increase in GCF levels.

Introduction

Orthodontic tooth movement (OTM) is based on force-induced periodontal ligament (PDL) and alveolar bone remodelling (Takahashi *et al.*, 2006). Mechanical stimuli exerted on a tooth cause an inflammatory response in the periodontal tissues, and biochemical changes occur such that the remodelling of the PDL space and surrounding tissues can take place (Cantarella *et al.*, 2006). The inflammatory mediators that trigger the biological processes associated with alveolar bone resorption and apposition are released (Basaran *et al.*, 2006). Of the myriad inflammatory mediators that are potentially involved in this process, matrix metalloproteinases (MMPs) have been implicated in OTM (Capelli Júnior *et al.*, 2011; Drummond *et al.*, 2011). The MMPs represent a family of proteases that play key roles in the remodelling of the ECM. Based on their molecular structures and substrate preferences, the MMPs are generally classified into several subgroups: collagenases (MMP-1, -8, and -13) that disintegrate native fibrillar collagens, gelatinases (MMP-2 and -9) that cleave denatured collagen, stromelysins (MMP-3, -10, and -11), membrane-type MMPs (MMP-14, -15, -16, and -17), and a group of miscellaneous MMPs (van Beurden *et al.*, 2005; Takahashi *et al.*,

2006). MMPs are secreted as inactive proenzymes that can be activated by proteolytic processing in the ECM (van Beurden *et al.*, 2005; Cantarella *et al.*, 2006; Bildt *et al.*, 2009). The MMPs and their specific inhibitors—the tissue inhibitors of metalloproteinases (TIMPs)—act in a coordinated fashion to regulate the remodelling of periodontal tissue (Takahashi *et al.*, 2006).

Gingival crevicular fluid (GCF) carries a multitude of inflammatory mediators that are present in the periodontal tissues during its passage into the gingival sulcus. This easily collected sample has been used to study the levels of several molecules that are released during OTM in humans (Apajalahti *et al.*, 2003b; Ingman *et al.*, 2005; Tuncer *et al.*, 2005; Cantarella *et al.*, 2006; Dudic *et al.*, 2006; Bildt *et al.*, 2009; Capelli *et al.*, 2010; Drummond *et al.*, 2011). Most of these studies have confirmed the involvement of MMPs in the biological mechanism triggered by orthodontic forces (Ingman *et al.*, 1996, 2005; Apajalahti *et al.*, 2003a,b; Cantarella *et al.*, 2006; Bildt *et al.*, 2009). This study considers the hypothesis that during OTM an increase in the expression levels of MMPs in GCF occurs since they are involved in different phases of remodelling of collagen. Therefore, this study aimed to determine changes in the

MMP levels (MMP-1, -2, -3, -7, -8, -12, and -13) in the GCF undergoing orthodontic treatment.

Subjects and methods

Subject population and study design

GCF samples were collected from 16 subjects (9 males and 7 females; 17.7 years of age, ranging from 13 to 27 years) applying for orthodontic treatment. The following inclusion criteria were followed: need for fixed appliance therapy involving extraction of the first upper premolars and distal retraction of the maxillary canines and good general health and no use of anti-inflammatory drugs in the month preceding the beginning of the study. Exclusion criteria were autoimmune diseases, pregnancy, lactation, and use of any medication that could interfere in OTM, which had adverse effects directly on the periodontium or interfered with the inflammatory process, up to 6 months before the start of the study (e.g. antibiotics, antihistamines, cortisone, immunosuppressants, and hormones). The subjects were informed of the characteristics and objectives of this study and signed or, if the subject was a minor, had a responsible adult sign an informed consent form (1948-CEP/HUPE). The protocol was approved by the Dental School of State University of Rio de Janeiro, Rio de Janeiro, Brazil. The study was approved by the local medical ethics committee. A clinical exam—including the plaque index, any bleeding upon probing and the probing depth—was performed by a periodontist in all of the subjects and authorized them to participate in project in light of the inexistence of gingivitis or other periodontal diseases. All of the subjects were treated in the orthodontics department and, at the beginning of the study (7 days before introducing orthodontic forces), received oral hygiene instructions regarding how to use a toothbrush and dental floss. They were also asked to use a 0.12% chlorhexidine gluconate mouth rinse twice daily for 4 consecutive weeks as an adjunct to oral home care.

The premolars were extracted at least 20 days prior to the retraction of the canines. The maxillary arch of each patient was divided into a test side (with orthodontic force introduction) and a control side (without force application) (Figure 1). This choice was randomly made during the study period. On the test side, a NiTi coil spring (Morelli, Sorocaba, Brazil) was used to move upper cuspids distally. The NiTi closed coil spring was mounted between a 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. No orthodontic appliance was placed on the mandibular arch during this visit or throughout the study period. After the experiment, the treatment continued with force application on both sides. The subjects were instructed not to use anti-inflammatory drugs during the study.

GCF sampling

Before GCF collection, any supragingival plaque was removed with cotton pellets, and a gentle air stream was directed towards the tooth surface for 5 seconds to dry the area. The GCF was then collected with standardized sterile paper strips (Periopaper®, Interstate Drug Exchange). The paper strip was inserted into the crevice until mild resistance was felt, and it was left in place for 30 seconds (Figure 2). Any strips that were visually contaminated with saliva or blood were discarded. The GCFs of the test and control teeth were sampled from the mesial and distal faces of the upper canines of each patient at seven specific times, according to the following schedule: 7 days before applying the orthodontic force; immediately before applying the orthodontic force; and after 1 hour, 24 hour, and 7, 14, and 21 days, respectively, called -7 days, 0 hour, 1 hour, 24 hour, 7 days, 14 days, and 21 days. The GCF volumes were determined using a pre-calibrated Periotron 8000™ (Oraflow Inc.). The paper strips were immediately placed into individually sealed plastic tubes (Eppendorf®), snap-frozen in liquid nitrogen, and stored at -70°C until further processing was carried out.

The levels of MMP-1, -2, -3, -7, -8, -12, and -13 were determined using a multiplexed bead immunoassay. Five assays were performed using the Human 7-Plex MMP Fluorokine® MultiAnalyte Profiling kit (R&D Systems, Minneapolis, USA). The beads were analysed with the Luminex 100™ instrument (MiraiBio, USA).

Quantification of inflammatory mediators

The levels of MMP-1, -2, -3, -7, -8, -12, and -13 were determined using a multiplexed bead immunoassay. The assay was performed in a 96-well filter plate as previously described (Vignali, 2000). Briefly, the filter plate was pre-wetted with washing buffer, and the solution was aspirated from the wells using a vacuum manifold (Millipore Corporation, USA). Microsphere beads coated with monoclonal antibodies against different target analytes were added to the wells. Five assays were performed using the Human 7-Plex MMP Fluorokine® MultiAnalyte Profiling kit (R&D Systems, Minneapolis, USA).

Samples and standards were pipetted into the wells and incubated for 2 hour with the beads. The wells were washed using the vacuum manifold (Millipore Corporation), and biotinylated secondary antibodies were added. After incubation for 1 hour, the beads were washed followed by an incubation of 30 minutes with streptavidin conjugated to the fluorescent protein, R-phycoerythrin (streptavidin-RPE). After washing to remove the unbound streptavidin-RPE, the beads (minimum of 100 per analyte) were analysed in the Luminex 100™ instrument (MiraiBio, USA). The concentrations of the unknown samples (antigens in GCF samples) were estimated from the standard curve and expressed as pg per site.



Figure 1 The test side with orthodontic force application by coil-spring activation.



Figure 2 Gingival crevicular fluid (GCF) sampling with the paper strip in position.

Data analysis

The data available for each subject were the volume of GCF (μl) and the levels of MMP-1, -2, -3, -7, -8, -12, and -13 at four sites per subject at -7 days, 0 hour, 1 hour, 24 hours, 7 days, 14 days, and 21 days. These data were expressed as pg per site.

The groups (CT, TT, CP, and TP) were compared individually for each face over time and between times. The data evaluations of the MMPs in the GCF were performed using the median of each variable. To compare each MMP in the GCF in control tension group (CT) versus the test tension group (TT) and the control pressure group (CP) versus the test pressure group (TP), the Wilcoxon test was used because it uses paired samples with non-normal distributions. The Wilcoxon test was also used to compare the changes in the MMPs at each individual time in the different groups and compare the tension and pressure sides within each group. The comparisons over time were made using Friedman's test and Dunn's test for multiple comparisons.

Results

All 16 subjects completed the study. The expression levels of MMP-1, -2, -3, -7, -8, 12, and -13 in the GCF on the pressure and tension sides (both control and test) at all times are represented in Figures 3 and 4.

The Luminex analysis was efficient in identifying and determining the levels of all of the MMPs. On the CT side, we only observed a statistically significant change in MMP-1 ($P = 0.015$) over time. However, the *post hoc* statistical analysis was unable to identify differences between any specific times. On the CP side, the results reveal a change in the MMP-1 ($P = 0.001$) and MMP-3 ($P = 0.012$) data that were statistically significant only between 24 hours and 7 days and 7 and 21 days in the two groups. On the TT side the results showed a statistically significant change in MMP-3 ($P = 0.001$). Dunn's analysis showed that these differences were found between 24 hours and 7 days, 7 and 14 days, and 21 and 7 days. The results showed a statistically significant difference in MMP-1 ($P = 0.002$) and MMP-3 ($P = 0.008$) on the TP side. Dunn's analysis showed that these differences were found between 24 hours and 7 days for MMP-1 and between 7 and 14 days for MMP-3. We also assessed the differences between the groups (CT, CP, TT, and TP) at each time. These comparisons did not reveal any statistically significant differences. Similar comparisons between the pressure and tension sides at each individual time also revealed no statistically significant differences.

Discussion

Previous studies have suggested that GCF flow reflects changes in deeper periodontal tissues, such as the alveolar bone and the PDL of the teeth, while under the influence of orthodontic treatment (Grieve *et al.*, 1994; Heasman *et al.*, 1996; Iwasaki *et al.*, 2001, 2005; Tuncer *et al.*, 2005; Krishnan and Davidovitch, 2006; Masella and Meister, 2006).

It can be observed that orthodontic interventions cause tissue injuries and subsequent inflammatory responses. Elevated MMP expression levels are the result of both the mechanical stress and the inflammatory responses that occur when collagenases are highly expressed by the polymorphonuclear cells, macrophages, and fibroblasts that invade the injured area (Redlich *et al.*, 2001).

In this study, MMP-1, -2, -3, -7, -8, -12, and -13 were chosen because they are involved in different phases of collagen remodelling, and they catalyse the initial degradation of most of the PDL extracellular matrix. In this study, changes in the MMP levels over time and at sites subjected to orthodontic forces were examined. In addition, because the nature of the mechanical stress (tension versus pressure) can differentially influence the expression of MMPs (Apajalahti *et al.*, 2003b; Cantarella *et al.*, 2006;

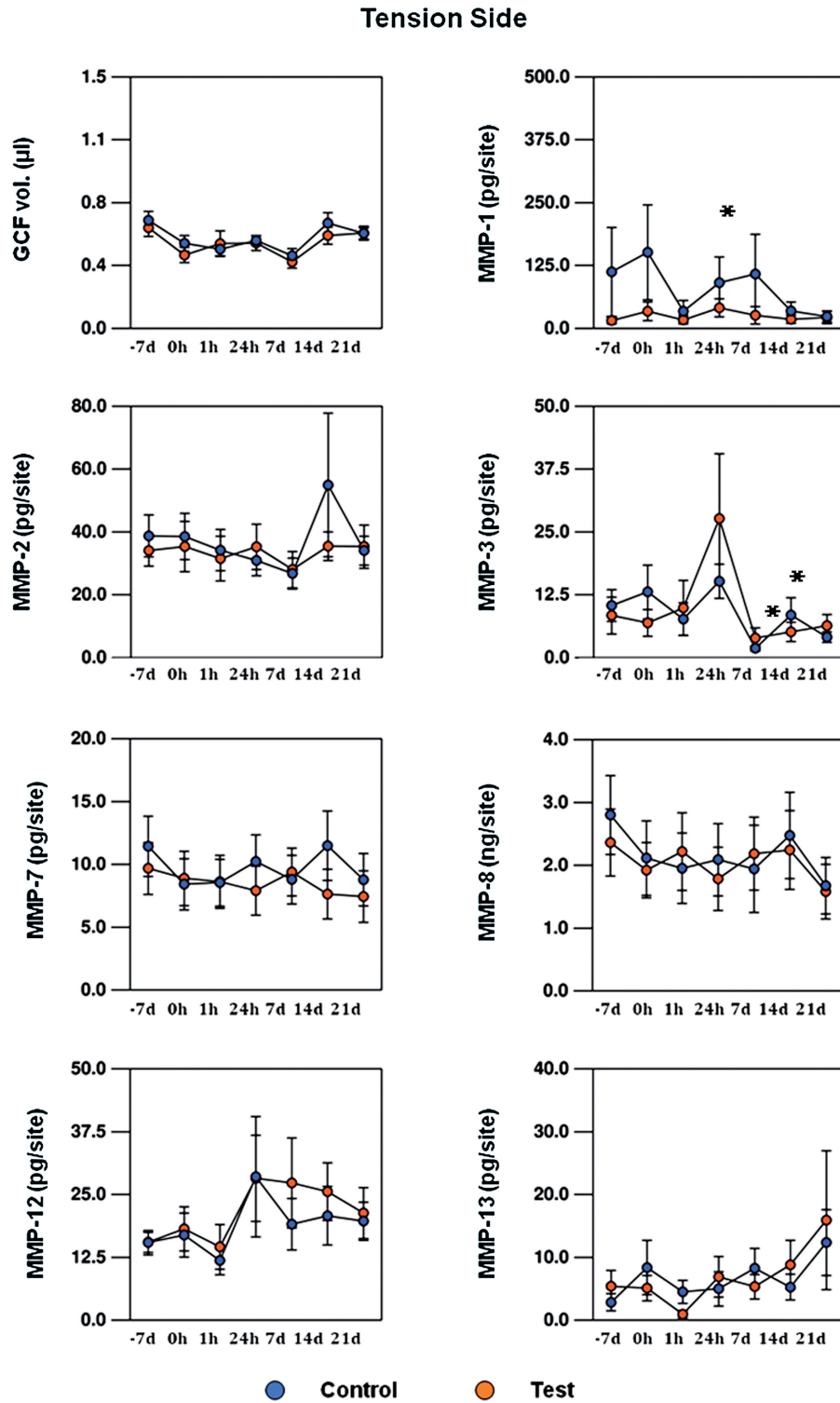


Figure 3 Fluctuations in the levels of GCF and matrix metalloproteinases (MMPs) -1, -2, -3, -7, -8, -12, and -13 (pg/site) on the tension side at different times during orthodontic movement (comparing the test and control sides). * $P < 0.05$.

Bildt *et al.*, 2009; Capelli *et al.*, 2010), the target analytes were compared between the tension and pressure sides of the moved teeth.

In vivo studies using GCF to evaluate MMP levels have produced contradictory results (Apajalahti, *et al.* 2003a, Ingman *et al.*, 2005; Cantarella, *et al.* 2006; Bildt *et al.*,

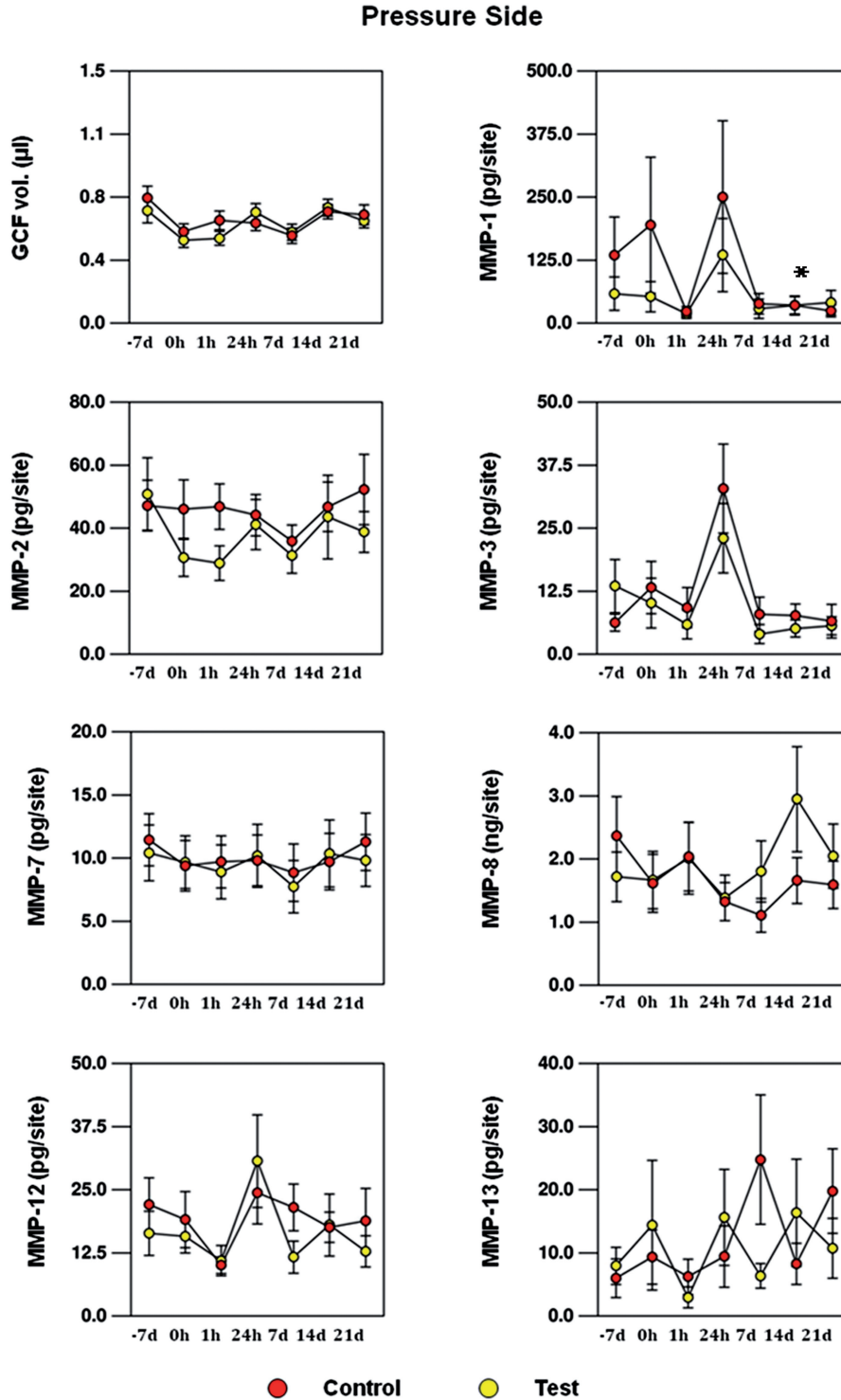


Figure 4 Fluctuations in the levels of GCF and MMP-1, -2, -3, -7, -8, -12, and -13 (pg/site) on the pressure side at different times during orthodontic movement (comparing the test and control sides). * $P < 0.05$.

2009). Nevertheless, most *in vitro* studies have demonstrated the up-regulation of MMP-1 and MMP-2 mRNA and protein production in human gingival and periodontal fibroblasts under mechanical stress (Carano and Siciliani,

1996; Bolcato-Bellemin *et al.*, 2000; Redlich *et al.*, 2001; Nahm *et al.*, 2004; Takahashi *et al.*, 2006).

The level of MMP-8 was much higher than that of the other MMPs (Figures 3 and 4). However, their variations were not

significant over time, both between the control and test sides and the pressure and tension sides. Thus, although it has the highest expression of MMP-8 found compared with the other MMPs, we cannot conclude that MMP-8 has collagenase activity in periodontal remodelling during OTM. In contrast, Apajalahti *et al.* (2003b) affirmed that the increased expression of MMP-8 reflects enhanced PDL remodelling activity induced by orthodontic movement. This increase in MMP-8 agrees with the pilot study by Ingman *et al.* (2005), which found that, when assessing patients daily for 30 days, the levels of this enzyme were significantly higher in orthodontic subjects than in the control group. However, Bildt *et al.* (2009) failed to identify MMP-8 in the GCF. Differences in the assessment methods, sampling times, and analysis techniques may partially explain the differences between this study and other studies reported in the literature.

Although other studies have demonstrated some increases in MMPs during OTM, no results that demonstrate a statistical pattern of the behaviours of these MMPs were found in this study (Apajalahti *et al.*, 2003b; Ingman *et al.*, 2005; Cantarella *et al.*, 2006). In some animals (mostly rats), experimental OTM was able to induce an observable increase in the expression of MMP-1, -2, -8, -9, and -13 and TIMP-1 and -3 in the PDL and alveolar bone (Domon *et al.*, 1999; Takahashi *et al.*, 2003, 2006). MMP-1 was also increased in the gingiva during tooth movement in dogs (Redlich *et al.*, 2001; Bildt *et al.*, 2009). Bolcato-Bellemin *et al.* (2000) found that MMP-1, -8, and -13 were expressed in the PDL, and their expression levels are transiently increased during OTM. Apajalahti *et al.* (2003b) reported that MMP-2 and -8 (and sometimes MMP-1) are increased in the GCF of orthodontic patients.

Differences in the methods and the time scale of MMP detection may, at least in part, explain the differences between the results of this study and those presented in the literature. The inconsistencies in the results may be attributed primarily to the different experimental conditions and models, such as the magnitude, duration and type of force, and the complex nature of the *in vivo* conditions (Redlich *et al.*, 2001).

To avoid any confounding factors, each patient in our study had his upper canines undergoing distal movement, and a fixed orthodontic appliance was used on both the test and its contralateral tooth (which represented the control tooth), as suggested by previous studies (Uematsu *et al.*, 1996; Cantarella *et al.*, 2006).

In this study, the levels of MMP-1 were slightly increased at some evaluated times, whereas the MMP-2 levels stayed constant, contradicting the findings of other studies that observed an increase in MMP-2 and the presence of higher levels of activated MMP-1 on the pressure and tension sides compared with the control side. However, other studies that aimed to evaluate the changes in MMP-1 during initial tooth movement were unable to detect the presence of MMPs in the GCF (Apajalahti *et al.*, 2003a; Ingman *et al.*, 2005).

MMPs play roles in the breakdown of collagen and ECM and PDL remodelling, which are important factors for orthodontic movement. Given that the volume and composition of the GCF are directly influenced by inflammation-induced gingival plaque (Egelberg, 1966), the subjects participating in the study received oral hygiene instructions, used 0.12% chlorhexidine gluconate and were followed up during the study period to minimize the impact of gingival inflammation.

A major limitation of GCF studies is the small sample volume for analysis (typically less than 0.5 µl) that is obtained from healthy sites. The small volume of GCF limits the number of analytes that can be conveniently studied by traditional enzyme-linked immunosorbent assays. The introduction of the multianalyte microsphere assay allows the simultaneous quantification of several targets in a single assay. The Luminex analyses were efficient for detecting MMPs in the GCF, confirming other findings that have demonstrated the feasibility of assessing MMPs in the GCF.

Conclusion

MMP-1, -2, -3, -7, -8, -12, and -13 were detected in the GCF on the control and test sides and on the pressure and tension sides at all analysed times. The fluctuations of these MMPs revealed few significant changes across times and between sides. The few variations that were found focused on MMP-1 and -3. There were no significant differences between the groups (TT, TP, CT, and CP) at each time, and the comparisons between the pressure and tension sides at individual time revealed no significant differences.

Funding

The authors would like to thank FAPERJ (Research Support Foundation of the State of Rio de Janeiro; Brazil) for financial support (grant number:E-26/111.155/2011).

References

- Apajalahti S, Sorsa T, Ingman T 2003a Matrix metalloproteinase -2, -8, -9, and -13 in gingival crevicular fluid of short root anomaly patients. *European Journal of Orthodontics* 25: 365–369
- Apajalahti S, Sorsa T, Railavo S, Ingman T 2003b The *in vivo* levels of matrix metalloproteinase-1 and -8 in gingival crevicular fluid during initial orthodontic tooth movement. *Journal of Dental Research* 82: 1018–1022
- Basaran G, Ozer T, Kaya F A, Hamamci O 2006 Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics* 130: 7.e1–e6
- Bildt M M, Bloemen M, Kuijpers–Jagtman A M, Von den Hoff J W 2009 Matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid during orthodontic tooth movement. *European Journal of Orthodontics* 31: 529–535
- Bolcato–Bellemin A L, Elkaim R, Abehsera A, Fausser J L, Haikel Y, Tenenbaum H 2000 Expression of mRNAs encoding for alpha and beta integrin subunits, MMPs, and TIMPs in stretched human

- periodontal ligament and gingival fibroblasts. *Journal of Dental Research* 79: 1712–1716
- Cantarella G, Cantarella R, Caltabiano M, Risuglia N, Bernardini R, Leonardi R 2006 Levels of matrix metalloproteinases 1 and 2 in human gingival crevicular fluid during initial tooth movement. *Am J Orthod Dentofacial Orthop* 130: 568.e511–e566
- Capelli J Jr, Fidel R Jr, Figueredo C M, Teles R P 2010 Alteração no volume do fluido gengival durante a retração de caninos superiores. *Dental Press Journal of Orthodontics* 15: 52–57
- Capelli Junior J, Kantarci A, Haffajee A, Teles R P, Fidel R Jr, Figueredo C M 2011 Matrix metalloproteinases and chemokines in the gingival crevicular fluid during orthodontic tooth movement. *European Journal of Orthodontics* 33: 705–711
- Carano A, Siciliani G 1996 Effects of continuous and intermittent forces on human fibroblasts in vitro. *European Journal of Orthodontics* 18: 19–26
- Domon S, Shimokawa H, Matsumoto Y, Yamaguchi S, Soma K 1999 In situ hybridization for matrix metalloproteinase-1 and cathepsin K in rat root-resorbing tissue induced by tooth movement. *Archives of Oral Biology* 44: 907–915
- Drummond S, Canavaro C, Perinetti G, Teles R, Capelli J, Jr 2011 The monitoring of gingival crevicular fluid volume during orthodontic treatment: a longitudinal randomized split-mouth study. *European Journal of Orthodontics* 34: 109–113
- Dudic A, Kiliaridis S, Mombelli A, Giannopoulou C 2006 Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides. *European Journal of Oral Sciences* 114: 416–422
- Egelberg J 1966 Permeability of the dento-gingival blood vessels. 1. Application of the vascular labelling method and gingival fluid measurements. *Journal of Periodontal Research* 1: 180–191
- Grieve W G, Johnson G K, Moore R N, Reinhardt R A, DuBois L M 1994 Prostaglandin E (PGE) and interleukin-1 beta (IL-1 beta) levels in gingival crevicular fluid during human orthodontic tooth movement. *American Journal of Orthodontics and Dentofacial Orthopedics* 105: 369–374
- Heasman P A, Millett D T, Chapple I L 1996 *The periodontium and orthodontics in health and disease*, 1st edn. Oxford University Press, New York
- Ingman T, Apajalahti S, Mantyla P, Savolainen P, Sorsa T 2005 Matrix metalloproteinase-1 and -8 in gingival crevicular fluid during orthodontic tooth movement: a pilot study during 1 month of follow-up after fixed appliance activation. *European Journal of Orthodontics* 27: 202–207
- Ingman T *et al.* 1996 Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *Journal of Clinical Periodontology* 23: 1127–1132
- Iwasaki L R *et al.* 2005 Tooth movement and cytokines in gingival crevicular fluid and whole blood in growing and adult subjects. *American Journal of Orthodontics and Dentofacial Orthopedics* 128: 483–491
- Iwasaki L R, Haack J E, Nickel J C, Reinhardt R A, Petro T M 2001 Human interleukin-1 beta and interleukin-1 receptor antagonist secretion and velocity of tooth movement. *Archives of Oral Biology* 46: 185–189
- Krishnan V, Davidovitch Z 2006 Cellular, molecular, and tissue-level reactions to orthodontic force. *American Journal of Orthodontics and Dentofacial Orthopedics* 129: 469.e461–e432
- Masella R S, Meister M 2006 Current concepts in the biology of orthodontic tooth movement. *American Journal of Orthodontics and Dentofacial Orthopedics* 129: 458–468
- Nahm D S, Kim H J, Mah J, Baek S H 2004 In vitro expression of matrix metalloproteinase-1, tissue inhibitor of metalloproteinase-1 and transforming growth factor-beta1 in human periodontal ligament fibroblasts. *European Journal of Orthodontics* 26: 129–135
- Redlich M, Reichenberg E, Harari D, Zaks B, Shoshan S, Palmon A. 2001. The effect of mechanical force on mRNA levels of collagenase, collagen type I, and tissue inhibitors of metalloproteinases in gingivae of dogs. *Journal of Dental Research* 80: 2080–2084
- Takahashi I *et al.* 2003. Expression of MMP-8 and MMP-13 genes in the periodontal ligament during tooth movement in rats. *Journal of Dental Research* 82: 646–651
- Takahashi I, Onodera K, Nishimura M, Mitnai H, Sasano Y, Mitani H 2006 Expression of genes for gelatinases and tissue inhibitors of metalloproteinases in periodontal tissues during orthodontic tooth movement. *Journal of Molecular Histology* 37: 333–342
- Tuncer B B *et al.* 2005 Levels of interleukin-8 during tooth movement. *The Angle Orthodontist* 75: 631–636
- Uematsu S, Mogi M, Deguchi T 1996 Interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. *Journal of Dental Research* 75: 562–567
- van Beurden H E, Snoek P A, Von den Hoff J W, Torensma R, Maltha J C, Kuijpers-Jagtman A M 2005 Dynamic protein expression patterns during intraoral wound healing in the rat. *European Journal of Oral Sciences* 113: 153–158
- Vignali D A 2000 Multiplexed particle-based flow cytometric assays. *Journal of Immunological Methods* 243: 243–255