

The level of cathepsin B in gingival crevicular fluid during human orthodontic tooth movement

Yuriko Sugiyama, Masaru Yamaguchi, Mika Kanekawa, Mizuho Yoshii, Tomomi Nozoe, Aki Nogimura and Kazutaka Kasai

Department of Orthodontics, Nihon University School of Dentistry at Matsudo, Chiba, Japan

SUMMARY This investigation examined gingival crevicular fluid (GCF) levels of lysosomal cysteine protease, cathepsin B (CAB), during human orthodontic tooth movement. The study included 10 patients (five males, mean age 22.5 ± 2.8 years and five females, mean age 23.4 ± 3.9 years), each having one tooth undergoing orthodontic movement, while the contralateral and antagonist teeth were used as the controls. The GCF was sampled at the control and treatment (compression) sites before activation and at 1, 24, and 168 hours. Prevention of plaque-induced inflammation allowed this study to focus on the dynamics of mechanically stimulated CAB levels in GCF. The CAB levels in GCF were determined by fluorospectrometry, using Z-Arg-Arg-MCA as the substrate and by Western blotting analysis.

The GCF levels of CAB for the treated teeth were significantly ($P < 0.001$) higher than those of the control teeth at 24 hours. At the control sites, CAB levels at 24 hours did not change significantly with time. At the experimental site where orthodontic forces were applied, Western blot analysis demonstrated that the molecular forms were 29 kDa mature enzymes.

These results indicate that the amount of CAB in GCF is increased by orthodontic tooth movement. This increased CAB may be involved in extracellular matrix degradation in response to mechanical stress.

Introduction

Biologically active substances, such as cytokines and enzymes, are expressed by cells within the periodontium in response to mechanical stress from orthodontic appliances (Davidovitch *et al.*, 1988; Saito *et al.*, 1991). The overall objective of many investigations has been to understand further the mechanisms for converting physical stress to the cellular responses resulting in tooth movement. In order to monitor the expression of biologically active substances non-invasively in humans, changes in the composition of gingival crevicular fluid (GCF) during orthodontic tooth movement have been studied (Griffiths *et al.*, 1998; Iwasaki *et al.*, 2001). These substances are involved in bone remodelling and produced by the periodontal ligament (PDL) cells in sufficient quantities to diffuse into the GCF. Grieve *et al.* (1994) reported that prostaglandin E and interleukin (IL)-1 β in GCF were elevated during orthodontic tooth movement. Uematsu *et al.* (1996a) also demonstrated that IL-1 β , IL-6, tumor necrosis factor (TNF)- α , and epidermal growth factor (EGF) were elevated in the GCF during such movement. Thus, the amount of these substances in that fluid apparently increases during tooth movement.

Cathepsin B (CAB) is a typical and well-characterized lysosomal cysteine protease, which is isolated from the tissues of many mammalian species (Eeckhout and Vaes, 1977). It has been reported to have collagenolytic

activity, degrading soluble monomeric collagen and insoluble polymeric collagen *in vitro* (Etherington, 1976), and to activate the procollagenase produced by mammalian tissues (Eeckhout and Vaes, 1977). Therefore, CAB is believed to be important in intercellular protein catabolism. Eisenhauer *et al.* (1983) reported that significant levels of CAB existed in periodontal crevicular fluid collected from gingivitis patients, and they concluded that CAB might play an important role in periodontal tissue catabolism. Chen *et al.* (1998) reported that the CAB was increased in GCF obtained from chronic periodontitis patients. It has also been reported that IL-6 increases CAB activity in human PDL cells (Yamaguchi *et al.*, 2000). Moreover, Sasaki and Ueno-Matsuda (1992) reported that CAB is involved in the formation of resorption lacunae by means of intra- and extra-cellular degradation of collagen and other non-collagenous matrix proteins of primary teeth. Therefore, CAB may be involved in periodontal metabolism in the degradation of organic bone matrix containing collagen fibres (Goto *et al.*, 1994). However, little information is available concerning the production of these modulators during orthodontic tooth movement in human subjects.

This study investigated the levels of CAB in GCF at 0, 1, 24, and 168 hours after the start of orthodontic treatment, by fluorospectrometry, using Z-Arg-Arg-MCA as the substrate and by Western blot analysis.

Subjects and methods

Experimental subjects

Ten adult orthodontic patients (five males, mean age of 22.5 ± 2.8 years and five females, mean age of 23.4 ± 3.9 years) were selected to participate in this study. These patients met the following criteria: (1) good general health; (2) lack of antibiotic therapy during the previous 6 months; (3) absence of anti-inflammatory drug administration in the month preceding the study; (4) periodontally healthy with generalized probing depths ≤ 3 mm and no radiographic evidence of periodontal bone loss; and (5) requirement of first premolar extraction and canine distal tooth movement as part of their orthodontic treatment plan. Informed consent of the subjects was obtained according to the protocol reviewed by the Board of Nihon University School of Dentistry at Matsudo.

Experimental design

For each subject, a canine undergoing distal movement was used as the experimental tooth, and the contralateral and antagonistic canines served as the controls. Orthodontic brackets were placed on both the treatment and control teeth. For the edgewise technique, 0.018×0.025 -inch slot bands and brackets (Tomy International Inc., Tokyo, Japan) were used. The canines were retracted with elastometric chains (Tomy International Inc.) on a 0.017×0.025 -inch rectangular wire (Tomy International Inc.).

The experimental canines were moved in a distal direction through an archwire using an elastic chain exerting an initial force of 250 g. The amount of movement for each tooth was measured with digimatic callipers. After impression-taking and production of working models, measurement was performed using an electronic digital caliper (MAX-CAL; micrometer MFG, Co., Ltd, Japan) with an accuracy of 0.01 mm. The error associated with measuring tooth movement was determined by 10 measurements. At the distal aspects of the experimental and control teeth, GCF was collected for subsequent analysis, and the following periodontal examinations were conducted: probing depth, presence or absence of plaque, and bleeding on probing. Collections and examinations were conducted immediately before activation and at 1, 24, and 168 hours after the initiation of tooth movement.

GCF collection

GCF was collected from the tension side, using the method of Uematsu *et al.* (1996a). Bone resorption occurs on the pressure side as a consequence of trauma-induced reactions in the PDL tissue, whereas on the tension side continuous bone apposition will be seen

resulting in a maintained PDL width (Rygh *et al.*, 1986). The examination and collection procedures were performed in a dental chair under good light. Prior to GCF collection, gingival inflammation was assessed by recording the colour of the gingivae and plaque was determined according to the index proposed by Silness and Loe (1964). Since plaque would contaminate GCF samples (Griffiths *et al.*, 1992), light deposits were removed with a periodontal probe, while a sickle scaler was utilized for heavier deposits. Following GCF collection, probing depth and attachment level were recorded, to ensure that periodontal breakdown had not occurred during the study. Bleeding following probing was noted as a further assessment of inflammation.

The GCF sampling was performed using the method of Offenbacher *et al.* (1986). GCF was collected from the experimental and control teeth. The teeth were gently washed with water, and the sites under study were isolated with cotton rolls (to minimize saliva contamination) and gently dried with an air syringe. Paper strips (Periopaper, Harco, Tustin, CA, USA) were carefully inserted 1 mm into the gingival crevice and allowed to remain there for 30 seconds (Figure 1). After a 1-minute interval, a second strip was placed at the same site. Care was taken to avoid mechanical injury. The volume of GCF in the periopaper was measured with a Periotron 8000 (Harco) that was calibrated with human serum. GCF collection for a fixed amount of time allowed for standardization of site

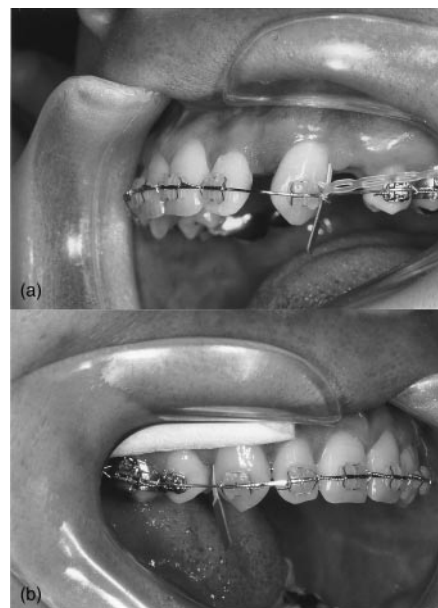


Figure 1 Collection of GCF. For each patient, GCF was sampled at the mesiofacial angle of the tooth undergoing labial movement, as well as at the contralateral and antagonist control teeth. (a) Experimental side; (b) contralateral side.

and subject differences. The paper strips from individual sites were stored at -30°C until further processing.

Assay of cathepsin B (CAB) activity

The assay of CAB activity was performed based on the method described by Chen *et al.* (1998). The strips were placed individually in 300 μl of 20 mM phosphate buffer, pH 6.0, containing 0.15 M NaCl, 1.0 mM EDTA, 1 mM dithiothreitol (DTT) and 0/1 per cent Tween 20. Samples were vortex mixed three times over a 30 minute period. The strips were removed, then the eluates were centrifuged for 5 minutes at 3000 g. The supernatants were separated and frozen at -30°C for later assay.

Protein concentration of the extract was estimated by the method of Bradford (1976), with bovine serum albumin as a standard. The levels of CAB in GCF were measured according to the method proposed by Barrett and Kirshchke (1981). Briefly, for measurement of CAB activity, 0.5 ml of the sample was added to 1.5 ml of buffer/activator (88 mM K_2PO_4 , 12 mM NaHPO_4 , 1.33 mM disodium EDTA, and 2.7 mM cystein, pH 6.0) and preincubated for 5 minutes at 40°C . After 50 μl of substrate solution (Z-Arg-Arg-MCA, dissolved in water at 20 mM) was added, the sample was mixed to start the reaction, which proceeded for precisely 10 minutes, then absorbance was read at 520 nm. One unit of enzyme activity was defined as the amount of enzyme required to release 1 mmol of product per minute under the assay conditions. CAB levels were shown as pU/ μl .

Western blotting analysis

The samples from GCF at 0, 1, 24, and 168 hours were examined. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) proteins were electrophoretically transferred to nitrocellulose paper as described by Towbin *et al.* (1979). The filter was soaked in buffer (50 mM Tris–HCl; 0.15 M NaCl, pH 7.3) containing 5 per cent low fat milk (Tris–milk) for 30 minutes and shaken gently. The strips of nitrocellulose paper were incubated with polyclonal antibody against CAB (Upstate Biotechnology, New York, USA; 1:200 dilution in Tris–milk,) for 4 hours at room temperature and then washed in goat anti-mouse IgG antibody (Upstate Biotechnology; 1:500 dilution on Tris–milk). The strips were again washed, and the reactive bands were visualized with H_2O_2 –chloronaphthol reagents.

Statistical methods

The values were calculated as the mean \pm standard deviation (SD), and statistical analysis was performed using the Statistica (V5.5, StatSoft Japan Inc., Tokyo, Japan) computer program. The Mann–Whitney U-test was used to compare the means of the groups.

Results

Clinical parameters

The amount of tooth movement was 1.2 ± 0.2 mm/168 hours on average, whereas no movement was detected for either of the control teeth. GCF volume has been correlated with the inflammatory state (Cimasoni, 1983). The mean volume of GCF from the experimental tooth at 24 hours (0.38 ± 0.04 μl /two periopapers) was slightly higher than that from control sites at 24 hours (0.37 ± 0.05 μl /two periopapers); however, there was no significant difference due to the great variation. In addition, the volume of GCF from around the experimental tooth was similar to that of the GCF samples (0.40 ± 0.05 μl /two periopapers) from healthy subjects. For all subjects, plaque accumulation was minimal throughout the entire study. Gingival health was excellent, with a lack of gingival bleeding. Probing depths were less than 2 mm at all times during the experiment.

The level of CAB in GCF

After orthodontic activation, significant differences were found between the two controls and the treated teeth for CAB values. The mean CAB values for the treated teeth were significantly higher than those of the two control sites at 24 hours (4.3 ± 0.7 versus 1.8 ± 0.4 and 1.5 ± 0.3 pU/ μl) (Figure 2).

Statistical analysis of the relationship between the treated site and both control sites, in terms of CAB activity, are summarized in Table 1. The Mann–Whitney U-test demonstrated that there were significant differences between treatment site and both control sites. There were no significant differences between the antagonistic and contralateral site.

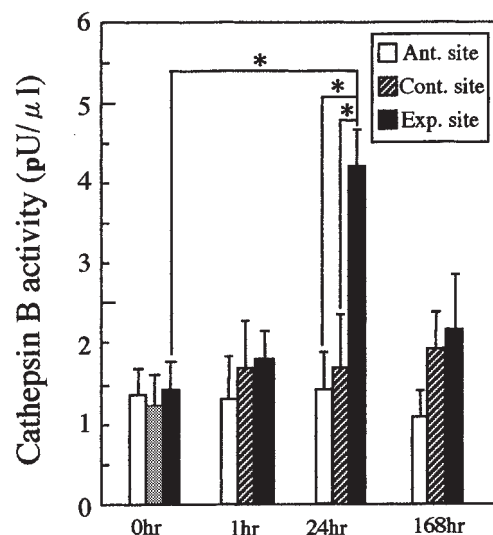


Figure 2 Alteration of CAB concentration in GCF during orthodontic tooth movement. * $P < 0.001$.

Table 1 Statistical analysis of CAB activity in the treated and control sites (antagonistic and contralateral sites).

	Mann-Whitney
Treated (0 h) versus treated (24 h)	0.004443*
Antagonist (24 h) versus Cont. (24 h)	0.705566†
Antagonist (24 h) versus treated (24 h)	0.004155*
Treated (24 h) versus Cont. (24 h)	0.003833*

*Significant difference. †Not significantly different.

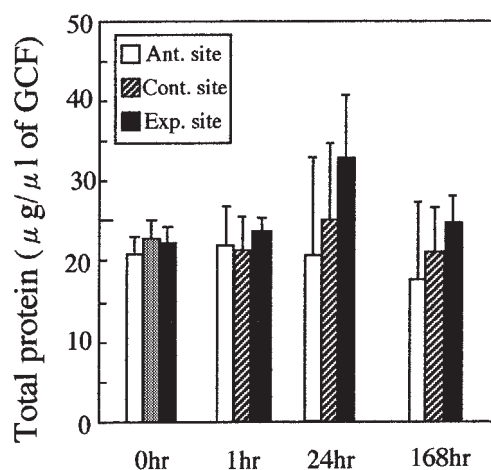
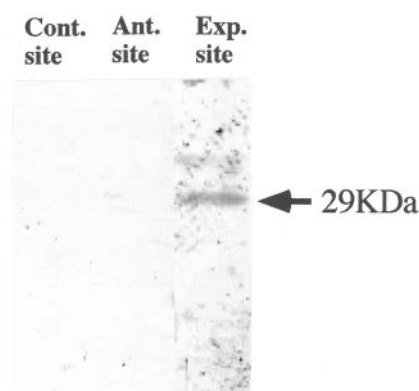
In contrast to the change in enzyme levels, there was no significant difference in total protein level over time (Figure 3). There was no significant difference between the experimental site at 24 hours and at baseline (34.0 ± 7.5 versus 25.3 ± 10.5 $\mu\text{g}/\mu\text{l}$), and also no difference between the experimental site at 24 hours and the antagonistic control site (34.0 ± 7.5 versus 22.1 ± 13.0 $\mu\text{g}/\mu\text{l}$). Therefore, the protein pattern was clearly different from the CAB pattern during orthodontic tooth movement.

Western blot analysis

CAB was increased in the GCF at 0, 1, 24, and 168 hours after tooth movement on Western blotting analysis. At the experimental site, where orthodontic force was applied, the levels of secreted CAB (29 kDa) were higher than the corresponding control sites at 24 hours. However, the control sites did not secrete detectable CAB at 0, 1, and 168 hours (Figure 4).

Discussion

The biochemical analysis of GCF shows promise as an effective means for monitoring and early detection of periodontal disease (Lamster *et al.*, 1988a,b). This fluid is

**Figure 3** Alteration of total protein content in GCF during orthodontic tooth movement.**Figure 4** Western blotting analysis of CAB in GCF during orthodontic tooth movement. The extract was separated under reduced condition on SDS-polyacrylamide gel (12.5 per cent) electrophoresis and then subjected to Western blotting analysis with anti-human CAB rabbit IgG. Staining with CAB antibody was demonstrated by horseradish peroxidase conjugated with secondary antibody to the rabbit IgG.

an osmotically mediated inflammatory exudate that is found in the gingival sulcus. As an exudate, the amount of fluid in any crevicular location tends to increase with inflammation and capillary permeability (Brill and Bjorn, 1959).

Orthodontic forces induce the movement of PDL fluids and, with them, any cellular biochemical product produced from prior mechanical perturbation. The direction of flow of the PDL fluid may be as follows: from an area of compression, to an area of tension, both apically and coronally, toward the gingival sulcus, and/or into the alveolar marrow spaces. Therefore, compression of the PDL should cause cellular biochemical by-products to appear in the sulcus. In addition, the effect of orthodontic force on the PDL is rapid, because the migration of carbon particles from capillaries in the compressed PDL occurs within minutes of the application of force to the guinea pig incisor (Storey, 1973).

As a result of the application of mechanical forces, PDL cells may produce sufficient amounts of modulators during bone remodelling to diffuse into the GCF. Some authors have reported that modulators such as PGE, IL-1 β (Grieve *et al.*, 1994), IL-1 receptor antagonist (Iwasaki *et al.*, 2001), IL-6 (Uematsu *et al.*, 1996a), TNF- α (Lowney *et al.*, 1995), transforming growth factor- β (Uematsu *et al.*, 1996b), and alkaline phosphatase (Insoft *et al.*, 1996) secreted in GCF are elevated during orthodontic tooth movement. Therefore, it is speculated that the elevation of cytokines and enzymes in the GCF reflects these comprehensive biological responses induced by mechanical stress.

Cathepsins are major degradative enzymes for long-lived proteins such as extracellular matrices and cell-surface receptors (Glaumann and Ballard, 1987) and 90 per cent of long-lived cellular proteins are speculated to

be degraded by lysosomal enzyme (Bohley and Seglen, 1992). Previous studies have suggested a relationship between periodontal disease and cathepsin activity. Eley and Cox (1991) reported that CAB activity in GCF correlated positively with clinical parameters of disease severity in untreated chronic periodontitis patients. These authors also found that following periodontal treatment, there were reductions in all clinical parameters and their protease activities (Cox and Eley, 1992). Therefore, this enzyme may be involved in the progression of periodontal disease.

It was hypothesized that this enzyme is produced by cells within the PDL and alveolar bone, such as fibroblasts, macrophages, osteoblasts, and osteoclasts. Mechanically deformed osteoblasts and PDL cells display a wide range of molecular alterations, some of which are capable of causing bone resorption (Sandy *et al.*, 1989) and extracellular matrix degradation (Yamaguchi *et al.*, 1997). Goseki *et al.* (1996) found that CAB activity increased with the progression of *in vitro* cellular ageing in PDL cells. Yamaguchi *et al.* (2000) have reported that IL-6 increased the CAB activity in human PDL cells *in vitro*. Therefore, PDL cells must play an important role in the production of this enzyme. Further studies are necessary to determine whether or not other cells also activate the level of CAB.

The finding of an increased level of CAB in the GCF adjacent to teeth undergoing orthodontic tooth movement indicates that cells within the periodontum are producing increased CAB in response to orthodontic force and that these mediators can be detected non-invasively in the GCF (Figure 2). The level of CAB in treated teeth was increased compared with control teeth at 24 hours. Uematsu *et al.* (1996a,b) also found that the concentrations of cytokines were increased compared with those at the control sites at 24 hours. Western blot analysis also showed that the levels of CAB (29 kDa) obtained from the experimental sites were higher than those obtained from the control sites (Figure 4). CAB is initially synthesized as a 41 kDa proform enzyme which is then processed to the 29 kDa mature single chain form (Kominami and Katunuma, 1989).

There are few investigations on the role of cathepsin during orthodontic tooth movement. Recent studies have reported that the mRNA expression of cathepsin K (CAK), a family of cysteine proteases such as CAB, was detected in the osteoclasts on the pressure side of the alveolar bone during experimental tooth movement in rats (Domon *et al.*, 1999; Ohba *et al.*, 2000). These results suggest that CAK is crucially involved in bone resorption during orthodontic tooth movement. CAK is thought to be specifically expressed in osteoclasts and plays an important role in osteoclastic bone resorption. However, as it has been reported that PDL cells express CAB and not CAK (Goseki *et al.*, 1996; Nishimura *et al.*, 2000), this investigation focused on the expression of CAB.

Another study suggests that CAB stimulates osteoclast-mediated bone resorption (Ishibashi *et al.*, 1999). Therefore, CAB may also be involved in bone resorption during orthodontic tooth movement.

Conclusions

1. The amount of CAB in GCF is increased by orthodontic tooth movement.
2. The increase in CAB may be involved in extracellular matrix degradation as a response to mechanical stress.

Address for correspondence

Masaru Yamaguchi
Department of Orthodontics
Nihon University School of Dentistry at Matsudo
2-870-1 Sakaecho-Nishi
Matsudo City
Chiba 271-8587
Japan

Acknowledgement

This research was supported in part by Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (c: 14571969).

References

- Barrett A J, Kirshchke H 1981 Cathepsin B, Cathepsin H, and Cathepsin L. *Methods in Enzymology* 80: 535–561
- Bohley P, Seglen P O 1992 Proteases and proteolysis in the lysosome. *Experientia* 48: 151–157
- Bradford M M 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254
- Brill N, Bjorn H 1959 Passage of tissue fluid into human gingival pockets. *Acta Odontologica Scandinavica* 17: 11–16
- Chen H Y, Cox S W, Eley B M 1998 Cathepsin B, α_2 -macroglobulin and cystatin levels in gingival crevicular fluid from chronic periodontitis patients. *Journal of Clinical Periodontology* 25: 34–41
- Cimasoni G 1983 Crevicular fluid updated. In: Myers H M (ed.) *Monographs in oral sciences*, Karger, Basel, pp. 1–152
- Cox S W, Eley B M 1992 Cathepsin B/L-, elastase-, trypsin-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid. *Journal of Clinical Periodontology* 19: 333–339
- Davidovitch Z, Nicolay O R, Ngan P W, Shanfeld J L 1988 Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. *Dental Clinics of North America* 32: 411–435
- 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
- Eeckhout Y, Vaes G 1977 Further studies on the activation of procollagenase, the latent precursor of bone collagenase. *Biochemical Journal* 166: 21–31

- Eisenhauer D A, Hutchinson R, Javed T, McDonald J K 1983 Identification of a cathepsin B-like protease in the crevicular fluid of gingivitis patients. *Journal of Dental Research* 62: 917–921
- Eley B M, Cox S W 1991 Cathepsin B- and L-like activities at local gingival sites of chronic periodontitis patients. *Journal of Clinical Periodontology* 18: 499–504
- Etherington D J 1976 Bovine spleen cathepsin B1 and collagenolytic cathepsin. A comparative study of the properties of the two enzymes in the degradation of native collagen. *Biochemical Journal* 153: 199–209
- Glaumann H, Ballard F J 1987 Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* 60: 849–859
- Goseki T, Shimizu N, Iwasawa T, Takiguchi H, Abiko Y 1996 Effects of *in vitro* cellular aging on alkaline phosphatase, cathepsin activities and collagen secretion of human periodontal ligament derived cells. *Mechanisms of Ageing and Development* 91: 171–183
- Goto T 1994 Localization of cathepsins B, D, and L in the rat osteoclast by immuno-light and -electron microscopy. *Histochemistry* 101: 33–40
- Grieve III W G, Johnson G K, Moor R N, Reinhardt R A, DuBois L M 1994 Prostaglandin E (PGE) and interleukin-1 β (IL-1 β) levels in gingival crevicular fluid during human orthodontic tooth movement. *American Journal of Orthodontics and Dentofacial Orthopedics* 105: 369–374
- Griffiths G S, Wilton J M, Curtis M A 1992 Contamination of human gingival crevicular fluid by plaque and saliva. *Archives of Oral Biology* 37: 559–564
- Griffiths G S, Moulson A M, Petrie A, James I T 1998 Evaluation of osteocalcin and pyridinium crosslinks of bone collagen as markers of bone turnover in gingival crevicular fluid during different stages of orthodontic treatment. *Journal of Clinical Periodontology* 25: 492–498
- Insoft M, King G J, Keeling S D 1996 The measurement of acid and alkaline phosphatase in gingival crevicular fluid during orthodontic tooth movement. *American Journal of Orthodontics and Dentofacial Orthopedics* 109: 287–296
- Ishibashi O, Mori Y, Kurokawa T, Kumegawa M 1999 Breast cancer cells express cathepsins B and L but not cathepsins K or H. *Cancer Biochemistry Biophysical* 17: 69–78
- Iwasaki L R, Haack J E, Nickel J C, Reinhardt R A, Petro T M 2001 Human interleukin-1 β and interleukin-1 receptor antagonist secretion and velocity of tooth movement. *Archives of Oral Biology* 46: 185–189
- Kominami E, Katunuma N (eds) 1989 Biosynthesis, processings and localizations of lysosomal cysteine proteinases. In: *Intracellular proteolysis*. Japan Scientific Societies Press, Tokyo, pp. 52–60
- Lamster I B *et al.* 1988a Enzyme activity in crevicular fluid for detection and prediction of clinical attachment loss in patients with chronic adult periodontitis: six month results. *Journal of Periodontology* 59: 516–523
- Lamster I B, Oshrain R L, Florello L, Celenti R S, Gordon J M 1988b A comparison of four methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. *Journal of Clinical Periodontology* 15: 317–352
- Lowney J J, Norton L A, Shafer D M, Rossomando E F 1995 Orthodontic forces increase tumor necrosis factor α in the human gingival sulcus. *American Journal of Orthodontics and Dentofacial Orthopedics* 108: 519–524
- Nishimura F *et al.* 2000 High glucose suppresses cathepsin activity in periodontal-ligament-derived fibroblastic cells. *Journal of Dental Research* 79: 1614–1617
- Offenbacher S, Odle B M, Van Dyke T E 1986 The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* 21: 101–112
- Ohba Y, Ohba T, Terai K, Moriyama K 2000 Expression of cathepsin K mRNA during experimental tooth movement in rat as revealed by *in situ* hybridization. *Archives of Oral Biology* 45: 63–69
- Rygh P, Bowling K, Hovlandsdal L, Williams S. 1986 Activation of the vascular system: a main mediator of periodontal fiber remodeling in orthodontic tooth movement. *American Journal of Orthodontics* 89: 453–468.
- Saito M, Saito S, Ngan P W, Shanfeld J, Davidovitch Z 1991 Interleukin-1 β and prostaglandin E are involved in the response of periodontal cells to mechanical stress *in vivo* and *in vitro*. *American Journal of Orthodontics and Dentofacial Orthopedics* 99: 226–240
- Sandy J R *et al.* 1989 Murine osteoblasts release bone-resorbing factors of high and low molecular weights: stimulation by mechanical deformation. *Bone and Mineral* 5: 155–168
- Sasaki T, Ueno-Matsuda E 1992 Immunocytochemical localization of cathepsins B and G in odontoclasts of human deciduous teeth. *Journal of Dental Research* 71: 1881–1884
- Silness J, L  e H 1964 Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* 22: 112–135
- Storey E 1973 The nature of tooth movement. *American Journal of Orthodontics* 63: 292–314
- Towbin H, Staehelin T, Gordon J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America* 76: 4350–4354
- Uematsu S, Mogi M, Deguchi T 1996a Interleukin(IL)-1 β , IL-6, tumor necrosis factor- α , epidermal growth factor, and β_2 -microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. *Journal of Dental Research* 75: 562–567
- Uematsu S, Mogi M, Deguchi T 1996b Increase of transforming factor- β_1 in gingival crevicular fluid during human orthodontic tooth movement. *Archives of Oral Biology* 41: 1091–1095
- Yamaguchi M 1997 Effect of tension-force on plasminogen activator activity from human periodontal ligament cells. *Journal of Periodontal Research* 32: 308–314
- Yamaguchi M, Ueda Y, Kasai K 2000 Stimulatory effect of interleukin-6 on both cathepsins B and L activities from human periodontal ligament cells. *Waves* 60: 167–171

Copyright of European Journal of Orthodontics is the property of Oxford University Press / UK and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.