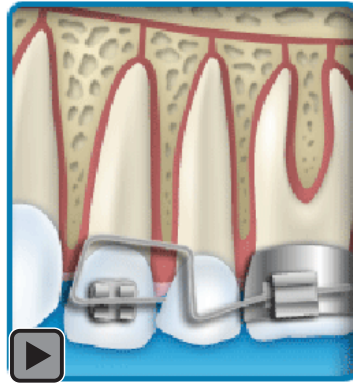


Bone Marker Levels in Gingival Crevicular Fluid During Orthodontic Intrusive Tooth Movement: A Preliminary Study

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Abstract

The aim of the present study is to observe the changes in bone turnover markers, deoxypyridinoline (Dpd), osteocalcin, n-telopeptide (NTx), and bone alkaline phosphatase (balp) during the experimental orthodontic intrusion of maxillary premolar teeth. The study population required fixed appliance therapy involving the extraction of the maxillary first premolar teeth. Gingival crevicular fluid (GCF) samples were collected from each patient by using paper strips before the appliances were fitted and 1, 24, and 168 hours after the activation of appliances. After the second activation on the 21st, 22nd, and 28th days of the study, samples were collected. Enzyme-Linked Immunosorbent Assay (ELISA) tests were performed following manufacturer's recommendations. The results of the study indicate Dpd, osteocalcin, and balp values decrease with force application. Among the tested parameters only Dpd values showed statistically significant changes through time. One, 7, 22, and 28 day results show a significant amount of decrease when compared to 0 days. The extra decrease on the 22nd day (the day after the second activation) is also significantly lower. NTx crosslink values could not be detected in the experimental samples.

Keywords: Intrusion, osteocalcin, bone alkaline phosphatase, deoxypyridinoline, n-telopeptide

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Introduction

When a small orthodontic force is applied to teeth for an adequate time period, an inflammatory event takes place in the periodontium resulting in bone remodeling that provides the movement of teeth. In order to develop biological strategies for enhancing this movement of teeth in bone, the underlying mechanisms of bone resorption and apposition should be understood in detail.

Analysis of gingival crevicular fluid (GCF) samples may be a good means of examining the ongoing biochemical processes associated with bone turnover during orthodontic tooth movement.¹⁻⁶ If it could be possible to biologically monitor and predict the outcome of orthodontic forces, then the appliance management could be based on individual tissue response and the effectiveness of the treatment could be improved. Additionally, the hard to solve retention problems could be addressed by monitoring the bone turnover rate present around the teeth. In this study bone alkaline phosphatase (balp) and osteocalcin, which are denoted as the best markers of bone formation in serum, and deoxypyridinoline (Dpd) and n-telopeptide crosslinks (NTx) – collagen degradation products in urine, which appear to be the most specific markers of systemic osteoclast activity, have been analyzed as bone markers in GCF samples.⁷



Dpd is excreted unmetabolized in urine and not affected by the diet, therefore, a very sound marker for resorption of bone in the body.⁸ In a study by Meng et al. pyridinium crosslinks have also been detected in the GCF of patients with disease active periodontal sites, but Griffiths et al. failed to show pyridinium crosslinks during orthodontic tooth movement in GCF.^{3,9} Type I collagen carboxyterminal telopeptide (ICTP) was studied in human GCF, and the results showed periodontal treatment decreased GCF ICTP concentration to the level seen in healthy subjects; however, large variations were seen between subjects and sites. ICTP values below

the detection limit were often found in deep pockets as well as high values in periodontitis-free subjects.¹⁰ In another investigation 24h total urine samples were collected in which the concentration of creatinine and collagen type I NTx were measured from pigs which were fitted with a mandibular protrusive orthodontic appliance.¹¹ It was concluded the protrusive appliances increased bone resorption significantly during the first two weeks of the trial. The assay was found sensitive enough to indicate changes in bone resorption, such as those caused by an orthodontic mandibular protrusive appliance.

Osteocalcin has been shown in the GCF of patients with periodontal disease; however, since these studies were cross-sectional, the association with active bone breakdown could not be determined.^{12,13} Other studies stated osteocalcin was present in GCF from both healthy and diseased periodontal sites.^{13,14} Similarly osteocalcin was detected during every stage of orthodontic movement, which may point out that it may merely be a constituent of GCF associated with the developing dentition and not suitable for being a marker for tooth movement.³

A peak of alkaline phosphatase between the first and third weeks of the experiment was reported by Insoft et al. who moved premolars buccally with 100 g of force and collected GCF weekly to assess for phosphatases.¹⁵ A recent investigation used a longitudinal design to investigate alkaline phosphatase activity in GCF and detected significantly elevated GCF alkaline phosphatase activity in the distalizing molars and the contralateral molars as compared with the antagonist molars at 1, 2, 3, and 4 weeks. Conversely, in the antagonist molars, GCF alkaline phosphatase activity remained at baseline levels throughout the experiment.¹⁶

The aim of the present study is to have an insight to the complex biological procedures taking place while teeth move in bone and detect the levels of these four bone markers: Dpd, cross-linked NTx of type I collagen, osteocalcin, and balp in the GCF, during intrusive tooth movement, which should mainly induce bone resorption.

Materials and Methods

Clinical Protocol and Sample Collection

The study population was randomly selected from patients who required fixed appliance therapy involving the extraction of the maxillary first premolar teeth. Patients included in the study (4 boys and 5 girls) had a mean age of 14.76 ± 2.08 years, and since bone loss can occur as a result of certain diseases or treatments, the patients were in good general and periodontal health clinically. An informed consent was obtained from each patient's parents before treatment. The study was approved by the Ethical Committee.

Brackets were bonded to both maxillary first premolars and intrusive springs were bent from 0.016×0.016 inch. TMA wires were tied to these brackets (Figure 1). A Nance appliance was fabricated for anchorage maintenance.

At the beginning and on the 21st day, the intrusive force of appliances were activated to be 50 g and measured by radial tension force gauge (Correx

Co, Bern, Switzerland). GCF samples were collected from the mesio-buccal, distobuccal, and palatal crevicular region of the maxillary first premolars (Figure 2).

These sites were isolated with cotton rolls and gently air dried. Any supragingival plaque was carefully removed prior to sampling. Three precut paper filter strips (Periopaper gingival fluid collection strips, ProFlow Inc., Amityville, NY, USA) were inserted with 5 sec intervals into the gingival crevice until mild resistance was felt and held in place for 60 sec. Samples were collected from each patient before the appliances were fitted and 1, 24, and 168 hours after the activation of appliances. On the 21st day of the study there was no sampling, only activation of the springs was done. After the second activation, samples were collected on the 22nd and 28th days. This sequence of collecting samples was repeated at the same time of the day, in the mid-morning hours, for all sampling time points for the entire group of patients. No effort was made to control for cyclic hormonal



Figure 1. The appliance - pre-activated and activated.



Figure 2. Filter paper strips in the crevicular region.

changes in female subjects. The samples (which belonged to right and left sides of the same patient on the same visit) were pooled in order to overcome problems rising from the smallness of the collected material. The patients were given 0.15% Benzidamin HCL mouth wash (Tantum Verde - A.C.R. Angelini F. Rome, Italy) in order to control any unwanted excessive gingival inflammation. The patients were given oral hygiene motivation at each appointment after GCF collection. Fluid volumes were measured by weighing the micro-centrifuge tubes with the precut paper filter strips for each site before and after sampling by using a Cahn 500 microbalance, accurate to the nearest 0.0001 g. Immediately after the measurement of fluid volume, a centrifugal elution technique was employed to recover the GCF samples from the paper strips. The paper strips from the individual sites were placed in sealed plastic micro-centrifuge tubes and centrifuged twice at 3000 rpm for 20 min by adding 50 µl of phosphate buffered saline (PBS) each time. The micro-centrifuge tubes were prepared beforehand by pushing small plastic caps halfway down the tubes. The caps were perforated in the middle in order to let the extract of the paper get to the bottom of the tubes when centrifuged. The extracts then were stored at -80°C until the enzyme linked immunoassay (ELISA) assays were carried out following the manufacturer's recommendation.

Biochemical Assay

ELISA kits (Metra Dpd EIA kit, catalog no: 8007, Quidel Corporation, San Diego, CA, USA) were utilized for the detection of Dpd, balp, and osteocalcin. Another ELISA kit by Osteomark

was used for NTx assay (Ostex International, Seattle, WA, USA). Each sample was analyzed in duplicate and compared to a standard curve. To correct for GCF collection, Dpd is expressed as pmol per mg GCF; osteocalcin and balp are expressed as amount or activity per mg or g GCF.

Statistical Method

Data management and statistical analysis of the present study were performed by using GraphPad Prism Version 3.0 software for Windows (GraphPad Software, Inc. San Diego, CA, USA, copyright 1994-1999). In evaluating the data, besides descriptive statistical methods means and standard deviation, the Friedman test was used for testing if the population means were equal. In order to determine which means are significantly different from each other Dunn's multiple comparison test was used. Ninety-five percent confidence interval and p<0.05 significance level were used while evaluating the results.

Results

Among the tested parameters, Dpd values showed statistically significant changes through time. Table 1 gives the results of the Friedman test; these values show a statistically significant change with respect to time during force application. The results of Dpd values for 1 hour, 1 day, and 7, 22, and 28 days, respectively, point out to a decreasing trend (Figure 3).

The decrease at 22 days (the day after the second activation) and at 28 days with respect to the initial force application are statistically significant (Table 2). Also, osteocalcin and

Table 1. Friedman test results.

	Osteocalcin	Balp	Dpd (pmol/mg GCF)
Initial	500.22±360.67	102.08±63.62	1.54±0.57
1 hour	477.18±277.17	91.82±65.07	1.16±0.66
1 day	451.86±330.07	57.39±33.48	0.95±0.56
7 days	505.85±236.15	75.14±49.56	0.85±0.50
22 days	326.98±240.58	44.60±24.17	0.52±0.12
28 days	309.22±173.85	52.35±27.10	0.75±0.48
F	9.69	4.61	18.44
p	>0.05	>0.05	<0.01*

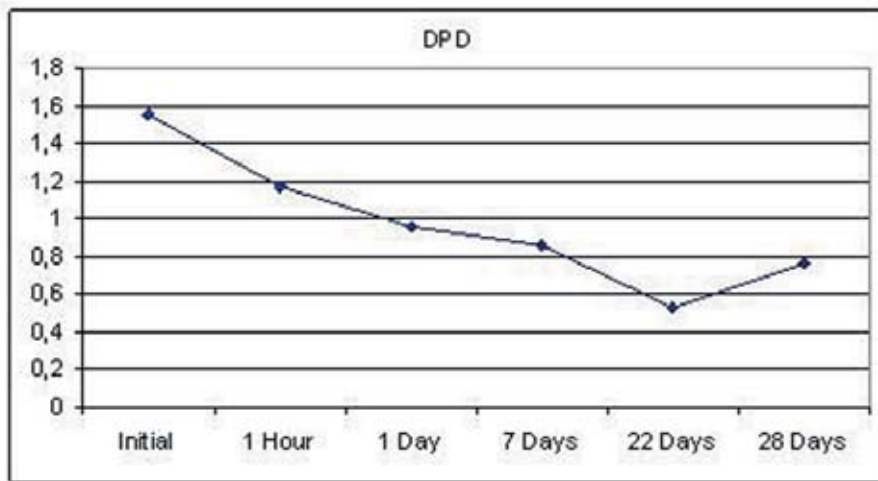


Figure 3. The changes seen in the Dpd values with respect to time intervals (pmol/mg GCF).

Table 2. Dunn's multiple comparison test results for Dpd.

Dunn's multiple comparison test	p
Initial / 1 hour	p > 0.05
Initial / 1 day	p > 0.05
Initial / 7 day	p > 0.05
Initial / 22 day	p < 0.01
Initial / 28 day	p < 0.05
1 hour / 1 day	P > 0.05
1 hour / 7 day	P > 0.05
1 hour / 22 day	P > 0.05
1 hour / 28 day	P > 0.05
1 day / 7 day	P > 0.05
1 day / 22 day	P > 0.05
1 day / 28 day	P > 0.05
7 day / 22 day	P > 0.05
7 day / 28 day	P > 0.05
22 day / 28 day	P > 0.05

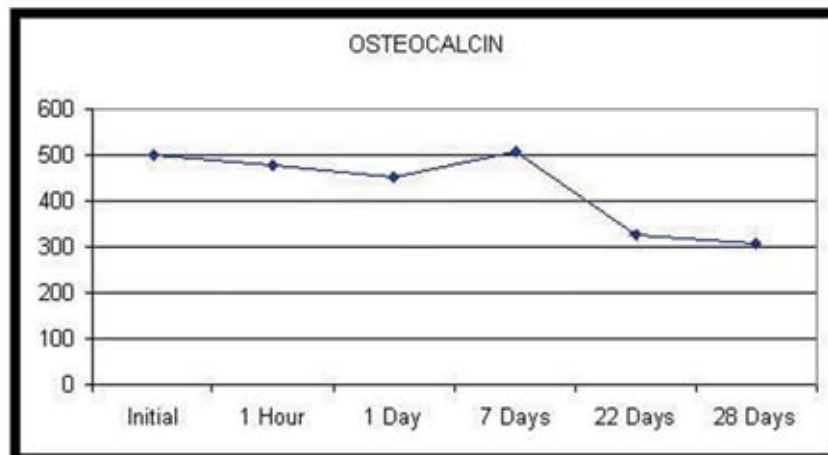


Figure 4. Time related changes of osteocalcin (pg/mg GCF).

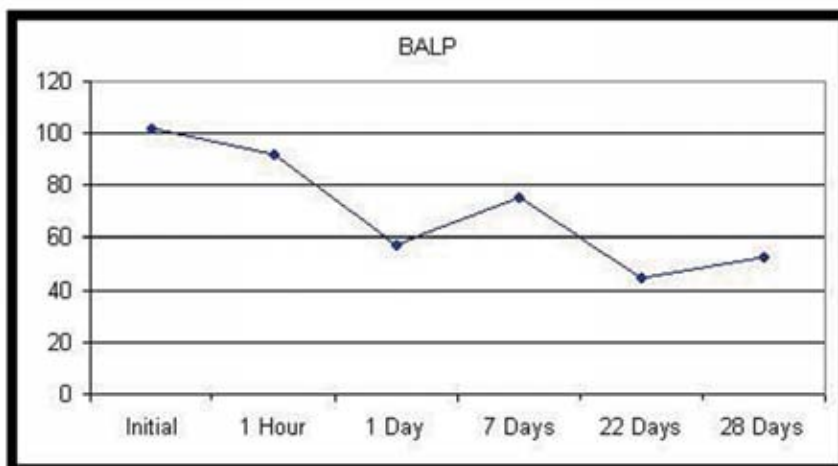


Figure 5. Time related changes of balp (mU/g GCF).

balp values show a descending character after activation visits (Figures 4 and 5), with the exception of the slight rise in the amount of markers on the 7th day of the experiment which all were found not to be statistically significant.

The Friedman test shows the results do not demonstrate any time dependent statistically significant changes in the amount of these two markers. NTx values were mostly found to be below the detection limit with a few readings which showed large variations between subjects and stages of tooth movement. For that reason, NTx measurements were not evaluated statistically.

The aim of the present study was to examine whether the amount of NTx, Dpd, osteocalcin, and balp alter during various stages of intrusive tooth movement in the GCF. Since the amount of bone markers and tooth movement are said to fluctuate during the day^{17,18}, sampling was carried out at the same time of the day – in the mid-morning hours. A 60 sec sampling time was selected for the present study. There are several protocols for tissue volume collection, like allowing the paper to remain in place until it was visibly wet just short of the calibration line, collecting the sample for a specific time period, or repeatedly sampling and discarding the first sample.^{19,20} One important drawback of long sampling time or not collecting the initial sample is the collected sample may contain plasma besides GCF.¹⁵ Some authors did not find this

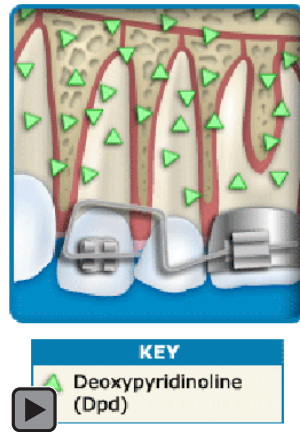
important because it is reported the measured levels of protein in GCF and plasma did not differ significantly, supporting the hypothesis GCF is an ultrafiltrate of plasma anyway.¹⁹ A statistically significant increase in plaque accumulation concurrent with the commencement of appliance therapy was observed in previous studies.^{3,21} In order to overcome this problem we motivated our patients at every visit and told them to use 0.15% Benzidamin HCL mouth wash in order to prevent any extra inflammation of the gingival tissues. As to the possible contamination of the filter paper from the small amount of plaque in the sulcus, it is suggested when supragingival plaque was added to standard concentrations of alkaline phosphatase there was no significant effect of the plaque on enzyme activity.¹⁹

The particular sampling points were chosen taking the three-week clinical visit intervals into consideration. After the first progressive displacement of the tooth relative to its osseous support, which takes about one week-apparently because of areas of periodontal ligament necrosis (hyalinization), a second phase of tooth movement occurs. The lag phase between these two stages of movement usually lasts two to three weeks.²²

The results of this study show Dpd, osteocalcin, and balp values begin to decrease with force application. Osteocalcin and balp graphics indicate a slight increase on the 7th day after the first activation (Figures 4 and 5). The first day after

the second activation also shows a dramatic decrease following with a slight recovery at the 28th day.

Griffiths et al.³, who also studied osteocalcin with a similar method, claimed osteocalcin may merely be a constituent of GCF associated with the developing dentition and this would reduce its potential to serve as a marker of bone turnover during tooth movement.



When we look at the studies carried out to reveal the association between osteocalcin levels of GCF and periodontal bone turnover, Lee et al.¹⁴ similarly did not find any differences in osteocalcin levels of healthy and diseased sites in subjects with adult periodontitis. On the contrary, osteocalcin concentration in GCF was shown to increase during the periods of maximum bone resorption during progressing experimental gingival disease²³, which would support the conclusions of earlier research.^{12, 13}

Our study points out statistically significant changes in the GCF Dpd values between the initial 22nd and initial 28th days (Table 2). These changes may represent a gradual decrease of Dpd parallel with the start of force application, a second decrease after the second activation, and the slight increase of the marker concentration in the 7th day of the second activation period. This result may be attributed to the intrusive force applied to the premolars, resulting in hyalinization of the surrounding tissues, which in turn slows down the bone turnover process taking place around the tooth. The bone resorption process may have started slightly again before the second activation, which again starts another phase of the hyalinization process. Only on the 28th day

of the experiment was there a slight increase in Dpd value (still lower than initial values), which may point out a slight resolution of the cell free status of the tissues. Although pyridinium (Ppd) crosslinks were shown to be detected in GCF of patients with disease active periodontal sites⁹, Griffiths et al.³ failed to show Dpd in any samples taken from teeth undergoing orthodontic tooth movement.

These authors have concluded either bone remodeling associated with orthodontic tooth movement may not generate Ppd or it may be confined in the tissues and not released. Another factor was considered to be the low sensitivity of the assay system due to the small volumes available, which may be true for all studies on GCF. NTx values of our study were mostly found to be below the detection limit with a few readings, which showed large variations between subjects and stages of tooth movement. A similar parameter in ICTP values below the detection limit have been found in deep pockets as well as high values in periodontitis-free subjects, pointing to large variations and inconsistencies between subjects and sites.¹⁰

Conclusion

Tooth intrusion can be used in order to study bone resorption and turnover. This study has shown a decrease of Dpd values in GCF at the 22nd and 28th days after the second activation (day 21) of intrusive springs on the maxillary first premolars. The applied forces may have caused hyalinization process and decrease of bone turnover. The mechanism should be mapped for tooth movement in all directions, in order to develop supporting medicament for more efficient and effective orthodontic tooth movement without having any associated adverse effects. Different force levels and longer follow up intervals should be considered in further study designs in order to have a better understanding of the bone resorption process during tooth intrusion.

References

1. Last KS, Donkin C, Embery G. Glycosaminoglycans in human gingival crevicular fluid during orthodontic movement. *Arch Oral Biol* 1988; 33 (12): 907-12.
2. Grieve WG 3rd, Johnson GK, Moore RN, Reinhardt RA, DuBois LM. Prostaglandin E (PGE) and interleukin-1 beta (IL-1 beta) levels in gingival crevicular fluid during human orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 1994; 105(4): 369-74.
3. Griffiths GS, Moulson AM, Petrie A, et al. Evaluation of osteocalcin and pyridinium crosslinks of bone collagen as markers of bone turnover in gingival crevicular fluid during different stages of ortodontic treatment. *J Clin Periodontol* 1995; 25: 492-498
4. Lowney JJ, Norton LA, Shafer DM, et al. Orthodontic forces increase tumor necrosis factor alpha in the human gingival sulcus. *Am J Orthod Dentofacial Orthop* 1995; 108(5): 519-24.
5. Uematsu S, Mogi M, Deguchi T. Increase of transforming growth factor-beta 1 in gingival crevicular fluid during human orthodontic tooth movement. *Arch Oral Biol* 1996; 41(11): 1091-5.
6. Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-1 beta, IL-6, tumor necrosis factoralpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. *J Dent Res* 1996; 75(1): 562-7.
7. Eyre DR, Bone biomarkers as tools in osteoporosis management. *Spine* 1997; 22(24S) Supplement: 17S-24S.
8. Colwell A, Russell RGG, Eastell R. Factors affecting the assay of urinary 3-hydroxypyridinium crosslinks of collagen as markers of bone resorption. *Eur J Clin Invest* 1993; 23: 341-349.
9. Meng XH, James IT, Skingle L, et al. Collagen cross-links in human gingival crevicular fluid (abstract #358). *J Dent Res* 1991; 70: 714.
10. Talonpoika JT, Hamalainen MM. Type I collagen carboxyterminal telopeptide in human gingival crevicular fluid in different clinical conditions and after periodontal treatment. *J Clin Periodontol* 1994 ;21(5):320-6.
11. Bollen AM, McCulloch KJ, Herring SW. Whole body bone resorption in the growing pig. *Growth Dev Aging* 1997; 61(3-4):181-9.
12. Kunimatsu K, Mataka S, Tanaka H, et al. A cross-sectional study on osteocalcin levels in givgival crevicular fluid from periodontal patients. *J Periodontol* 1993; 64: 865-869.
13. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E2 and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status. *J Clin Periodontol* 1994; 21: 327-333.
14. Lee AJ, Walsh TF, Hodges SJ, et al. Gingival crevicular fluid osteocalcin in adult periodontitis. *J Clin Periodontol* 1999; 26(4):252-6.
15. Insoft M, King GJ, Keeling SD. The measurement of acid and alkaline phosphatase in gingival crevicular fluid during orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 1996; 109(3): 287-96.
16. Perinetti G, Paolantonio M, D'Attilio M, et al. Alkaline phosphatase activity in gingival crevicular fluid during human orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2002; 122 (5): 548-56.
17. Miyoshi K, Igarashi K, Saeki S, et al. Tooth movement and changes in periodontal tissue in response to orthodontic force in rats vary depending on the time of day the force is applied. *Eur J Orthod* 2001; 23(4): 329-38.
18. Ladlow JF, Hoffmann WE, Breur GJ, et al. Biological variability in serum and urinary indices of bone formation and resorption in dogs. *Calcif Tissue Int* 2002; 70(3):186-93.
19. Binder TA, Goodson JM, Socransky SS. Gingival fluid levels of acid and alkaline phosphatase. *Journal of Periodontal Research* 1987; 22: 14-19.
20. Lamster IB, Harper DS, Goldstein S, et al. The effect of sequential sampling on crevicular fluid volume and enzyme activity. *J Clin Periodontol* 1989; 16: 252-258.
21. Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *J Periodontol* 1996; 67: 78-85.
22. Roberts WE. Bone physiology, metabolism, and biomechanics in orthodontic practice. In: *Orthodontics Current Principles and Techniques*. Graber TM, Vanarsdall RL Jr, editors. St.Louis, Missouri: Mosby Inc, 2000; 193-257.
23. Giannobile WV, Lynch SE, Denmark RG, et al. Crevicular fluid osteocalcin and pyridinoline cross-linked carboxyterminal telopeptide or type 1 collagen (ICTP) as markers of rapid bone turnover in periodontitis: a pilot study in beagle dogs. *J Clin Periodontol* 1995; 22: 903-910.

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