



# Osteocalcin expression in periodontal ligament when inducing orthodontic forces

## *Expresión de la osteocalcina en el ligamento periodontal al inducir fuerzas ortodóncicas*

Maritere Villarreal Brito,\* Marco Antonio Álvarez Pérez,<sup>§</sup> Francisco Javier Marichi Rodríguez<sup>||</sup>

### ABSTRACT

Osteocalcin is a non-collagenous protein located in alveolar bone, root cementum and subpopulations of periodontal ligament cells. This protein plays an important role in the biomineralization process and in the extra-cellular matrix, regulating maturation of hydroxyapatite and osteoclast recruitment which participate in bone remodeling. Periodontal tissue new formation and remodeling is a vital part of the process during orthodontic movements. These movements, when force is exerted, cause tension in the cells, provoking adaptation which results in molecular and cellular responses which, in turn, can affect the extracellular matrix. Due to the aforementioned facts, the aim of the present research was to determine osteocalcin expression associated to periodontal remodeling when orthodontic forces are applied. Roth 0.022 " fixed brackets with a NiTi 0.016" archwire were applied to first upper and lower bicuspid. This was applied to all teeth of both arches except to left lower and upper bicuspid. Bicuspid without brackets ( $t = 0$ ) as well as with brackets to elicit orthodontic movements during 1, 3, 5, 7 and 9 days were extracted to assess osteocalcin expression in the extra-cellular matrix of the periodontal ligament. The RT-PCR technique was followed to determine temporal and spatial expression of osteocalcin messengers. Osteocalcin expression in the experimental group was present in all test days, suggesting thus the fact that orthodontic movements elicit changes that are susceptible in osteocalcin protein messenger concentrations.

**Key words:** Osteocalcin, periodontal ligament, orthodontic forces.

**Palabras clave:** Osteocalcina, ligamento periodontal, fuerzas ortodóncicas.

### RESUMEN

La osteocalcina es una proteína no colágena presente en hueso alveolar, cemento radicular y subpoblaciones del ligamento periodontal. Esta proteína juega un papel importante en la biomineralización y en la matriz extracelular regulando la maduración de los cristales de hidroxiapatita y en el reclutamiento de los osteoclastos participando en la remodelación ósea. La remodelación y la nueva formación de tejido periodontal es parte esencial durante los movimientos ortodóncicos, los cuales al aplicar fuerzas causan tensión en las células provocando una adaptación que se traduce en respuestas celulares y moleculares que pueden afectar la matriz extracelular. Por ello, el propósito de esta investigación fue determinar la expresión de la osteocalcina asociada a la remodelación periodontal cuando se aplican fuerzas ortodóncicas. En primeros premolares superiores e inferiores se colocó aparatología fija prescripción Roth 0.022 con un arco NiTi 0.016, la cual se aplicó a todos los dientes de ambas arcadas con excepción de los premolares superiores e inferiores izquierdos. Los premolares sin aparatología ( $t = 0$ ) y en presencia de aparatología para inducir movimientos ortodóncicos durante 1, 3, 5, 7 y 9 días; fueron extraídos para analizar la expresión de la osteocalcina en la matriz extracelular del ligamento periodontal. Para determinar la expresión temporal y espacial de los mensajeros de la osteocalcina en el ligamento periodontal se llevó a cabo la técnica RT-PCR. La expresión de la osteocalcina en el grupo experimental estuvo presente en todos los días de prueba, sugiriendo que los movimientos ortodóncicos generan cambios que son susceptibles en las concentraciones del mensajero de la proteína osteocalcina.

### INTRODUCTION

Osteocalcin, or BGP (Bone Gla Protein) is the non-collagenous protein most abundant in the periodontium. It constitutes up to 3% of total bone protein. It contains 49 amino-acids with three gamma-carboxyglutamic acid residues, which are associated to its calcium-bonding properties. BGP is produced by

\* Orthodontics Program student, Graduate and Research School, National School of Dentistry.

§ Professor and Researcher, Molecular and Cellular Biology Laboratory, Graduate and Research School, National School of Dentistry.

|| Orthodontics Specialty Professor, Graduate and Research School, National School of Dentistry.

National University of Mexico (UNAM)

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osteoblasts, cementoblasts and periodontal ligament fibroblast subpopulations, totally differentiated with an 6kDa approximate mass. It is secreted at the mineralization site.<sup>1-3</sup>

Immuno-histochemistry studies have located BGP in alveolar bone, dentin and root cement. Its role in the biomineralization process has been partially determined. It is known that osteocalcin production is stimulated by vitamin K, since, in animal models treated with warfarin (vitamin K antagonist), inhibition of carboxylation of glutamic acid residues was observed, and to the type 2 (FGF-“) fibroblastic growth factor, since gene FGF-«knock out» mice presented bone hypermineralization. This suggested the fact that BGP plays a role in the biomineralization process as well as in the extracellular matrix by regulating hydroxyapatite cristal maturation and osteoclasts recruiting participating in bone remodelling.<sup>4-7</sup>

During orthodontic treatment, teeth respond to exerted mechanical forces by remodeling the extracellular matrix of the periodontium. When forces are exerted upon a tooth there are two main points which might influence (elicit) a response: a pressure side where resorption cycles occur, and a tension side, where extracellular matrix formation predominates. During the processes of resorption and periodontium extracellular matrix formation, under the influence of orthodontic movements, there is expression of a great number of molecules (growth factors, collagen molecules, glycoproteins, hormones, proteoglycans etc.) to regulate the functions of cellular adhesion, motility growth and differentiation. These functions are firmly regulated by the extracellular matrix and play a key role in orthodontic movements.<sup>8</sup>

Research conducted on orthodontic movements have reported that local application of osteocalcin produced acceleration of dental movement in rats during initial treatment phases. This acceleration was mainly due to an increase of osteoclast recruitment. This had led to suppose that osteocalcin application at initial stages can be sufficient to accelerate dental movement, since it could act as a molecule promoting chemo-attraction for osteoclasts precursor cells, participating thus in the remodeling of the matrix as well as favoring a suitable micro-environment for biomineralization.<sup>8,9</sup>

For all the aforementioned reasons, the aim of the present study was to determine the expression of the gene which codifies osteocalcin found in the periodontal ligament of teeth subjected to orthodontic forces during 1, 3, 5, 7 and 9 treatment days.

## METHODS

### PATIENT SELECTION AND FIXED APPLIANCES PLACEMENT

To conduct the present study, 50 patients were selected. Patients were healthy, of both genders, over 18 years of age, and attended the Orthodontics Clinic at the Graduate and Research Division of the National School of Dentistry, UNAM. Base treatment contemplated extraction of first upper and lower premolars. Patients were informed of the study and granted consent. The 50 patients were randomly divided into 10 patient groups to be allotted to each experimental period (1, 3, 5, 7 and 9 days). Roth 0.022" fixed brackets were placed with NiTi 0.016" gauge. Sentalloy archwire. Brackets were placed on all teeth of both arches, except for left upper bicuspid which acted as control group (t = 0). The study involved a split mouth design where right upper bicuspid were considered as the group subjected to orthodontic pressure (experimental group). To these bicuspid, a bracket was applied which exhibited a 30° distal angulation with respect to the patient's occlusal plane (*Figure 1*). This bracket was ligated to the archwire with an elastomeric module (GAC brand), and the tooth was subjected to gauged, constant orthodontic pressure during spans of 1, 3, 5, 7 and 9 days. Upon completion of each period in which orthodontic forces were applied, upper and lower bicuspid were extracted.

### DENTAL EXTRACTION

Patient premolar extraction was performed at the Periodontics Clinic of this same institution at intervals of 1, 3, 5, 7 and 9 days after bracket placement. Extractions were achieved with the help of 1.8 ml lidocaine and epinephrine anesthetic (36 mg/0.025 mg, Uniseal). Teeth were luxated with straight elevator Number 301 m and were manipulated with number 150 forceps. Extracted bicuspid were placed in liquid nitrogen for conservation and later use for RNA isolation.

### RNA ISOLATION

Bicuspid subjected to orthodontic movements (t = 1, 3, 5, 7 and 9 days) and not subjected to orthodontic movements (t = 0) were extracted in order to analyze osteocalcin gene expression present in the periodontium extra-cellular matrix. After bicuspid extraction, periodontal tissue bonded to bicuspid root's medial and apical zone was removed by scraping with

a scalpel blade. Tissues were homogenized in 1 mL TRIZOL (Gibco BRL) so as to conduct complete RNA extraction following manufacturer's indications. Total RNA was incubated with DNAase free of RNAase (Boehringer, Mannheim Biomedicals, IN) with the aim of guaranteeing absence of genomic DNA contamination. RNA was quantified with the help of 260nm optic densitometry and was later used for the RT-PCR technique.

### RT-PCR

RT-PCR technique was executed in order to determine spatial and temporary expression of osteocalcin messenger isolated from periodontal extracellular matrix subjected to orthodontic movements at 1, 3, 5, 7 and 9 days. Super-Script One-step RT-PCR kit was used (Invitrogen, Carlsbad CA) for transcription of the complementary chain from 1 µg of total RNA in each sample, following manufacturer's specifications. To generate each cDNA template, RT was achieved, osteocalcin specific oligo in sense - direction 5' GATCCATGAGAATGAGAAGCG 3' and direction-anti-sense 5' CTATGTTAGCACCTTATCCCC 3' at 45°C during 30 minutes, and a 94°C cycle for two minutes. cDNA was amplified under the following PCR program: 94°C during one minute ( de-naturalization), 55°C alignment temperature for the osteocalcin oligos during one minute, and extension to 72°C for one minute. This was performed until completing all 35 cycles. PCR products were separated with electrophoresis into 1.2% agarose gels. They were tinted with ethidium bromide and visualized under an ultraviolet light trans-illuminator. Images thus obtained were documented with EDAS 290 system (Kodak, USA).

### RESULTS

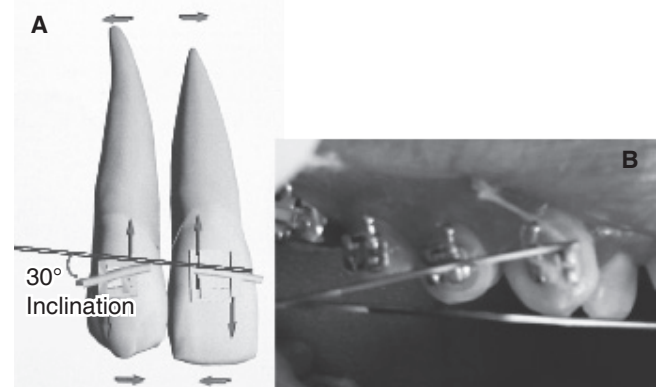
*Figure 1* shows a typical example of bicuspid which had been previously subjected to bracket placement (experimental site group) to induce orthodontic tension during 1, 3, 5, 7 and 9 days. The image shows the upper right bicuspid where 30° angulation with respect to occlusal plane can be appreciated. During the study of both groups: control group bicuspid, corresponding to zero time and experimental bicuspid, no noticeable clinical changes in periodontal ligament thickness could be observed during the time during which orthodontic forces were applied.

*Figure 2* shows the expression of the osteocalcin codifying gene which presents a molecular weight of approximately 310 bases pairs. Results obtained

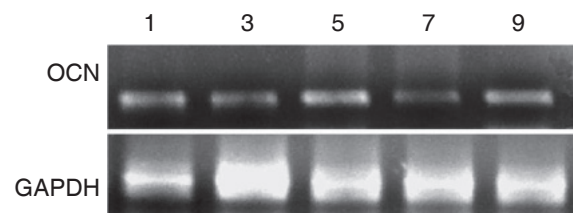
during the days when orthodontic forces were applied indicated that osteocalcin was expressed during all experimental times, and exhibited expression increase during treatment days five and nine. During treatment days 1, 3 and 7, expression was similar to time zero or control; day seven showed a slight decrease.

### DISCUSSION

Results obtained in the present study indicate the fact that when applying orthodontic forces with brackets to bicuspid during a span of one to nine days a change was elicited in the expression of the gene which codifies osteocalcin in the periodontal ligament area. Since osteocalcin plays a role in resorption as well as in the deposition of mineralized matrix, results obtained in the present study confirm the regulating role osteocalcin plays in the processes of periodontium extracellular matrixes. Expression of osteocalcin gene during experimental times can be attributed to induction of initial bone and matrix resorption of periodontal ligament collagen



**Figure 1.** Outline (A) and photograph (B) showing bracket placement with 30° distal angulation with respect to the patient's occlusal plane.



**Figure 2.** Expression of osteocalcin codifying gene at different days of orthodontic treatments. Internal control gene which codifies GAPDH.

fibers, which is needed for tooth movement and for osteoclasts specific recruitment at the resorption site. Other studies confirm the fact that in animal models, local application of protein increases speed of orthodontic movements.<sup>9</sup>

Expression of osteocalcin codifying gene kept a growing expression during the time of orthodontic movements. On the 5<sup>th</sup> day, an increase was observed; this suggested the fact that resorption maximum peak was being reached. On the 7<sup>th</sup> day a recessive phase took place, and in the 9<sup>th</sup> day a significant expression was observed. All the aforementioned would indicate the fact that a mineralization process was initiated, since there was a protein involved in regulating calcium and hydroxyapatite concentrations. Osteocalcin expression, as shown in *figure 2* hints that the matrix of the periodontal ligament can be remodeled from the first hours in which brackets are applied. Scientific literature counts with several reports demonstrating the effect of orthodontic forces on the periodontal ligament. Nevertheless, these studies have been conducted *in vitro* and/or in species different from human beings.<sup>10</sup>

These first response stages of the periodontal ligament can be related to the inflammation processes elicited when inducing tooth movement with orthodontic processes. Therefore, osteocalcin could facilitate orthodontic movements by acting as a molecule which induces chemo-attraction to recruit osteoclasts at sites of greater pressure; it can also act as a key factor helping bio-mineralization locations at the tension site.

### CONCLUSION

The present study suggested presence of changes at molecular level in the periodontal ligament matrix. These changes could be sensitive to movement induction time of the tooth when fixed appliances were used.

Molecular level changes were determined by the RT-PCR technique for the gene which codifies

osteocalcin. This technique reflected the fact that the periodontal ligament matrix was sensitive, in a given time, to the orthodontic forces exerted on the teeth used in the present study.

Osteocalcin expression suggested double participation in orthodontic processes: it took part in resorption as well as remodeling processes of the periodontal ligament matrix.

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Mailing address:

**Francisco Javier Marichi Rodríguez**

E-mail: fmarichi@fo.odonto.unam.mx