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# Biomarkers of Periodontal Tissue Remodeling during Orthodontic Tooth Movement in Mice and Men: Overview and Clinical Relevance

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Additional information is available at the end of the chapter

## Abstract

Tooth movement by orthodontic force application is dependent on remodeling in periodontal ligament and alveolar bone, involving the activation of complex cellular and molecular mechanisms correlated with several macro- and microscopic biological changes. The orthodontic process involves the activation of many complex cellular and molecular mechanisms mediated by the release of chemical substance cascades by many cells of the periodontium. Mainly during the early stage of application of orthodontic forces, an inflammatory process can occur in the periodontium as a physiological response to the tissue stress. Several potential biomarkers of the biological alterations after an orthodontic force application expressing bone resorption and formation, periodontal ligament changes, and vascular and neural responses, may be detected. The appropriate choice of the mechanical force to achieve the highest rate of tooth movement in the shortest time of treatment avoiding adverse consequences is a primary objective of a specialist. Thus, an insight into the biological phenomena occurring during the orthodontic therapies by evaluating these biomarkers may be quite relevant for the clinicians. In this chapter, two models of study, i.e., mice and men, were used to describe the clinical usefulness of some biomarkers in orthodontics.

**Keywords:** orthodontics, periodontal ligament, tooth movement, gingival crevicular fluid, biomarkers

## 1. Introduction

Tooth movement by orthodontic force application is dependent on remodeling in periodontal ligament (PDL) and alveolar bone, correlated with several macro- and microscopic biological changes.

After more than 100 years, since the first article on the theory of tooth movement was published by Carl Sandstedt (1904–1905), the specialists have reasonably good understanding of the sequence of events involved in the orthodontic tooth movement at tissue and cellular levels [1]. Orthodontic movements represent a continual and balanced process characterized by bone deposition in tension sites and bone resorption in the pressure ones. The mechanical stress from the application of orthodontic forces induces the activation of many cellular and molecular mechanisms mediated by the release of chemical substance cascade allowing the transmission of signals from extracellular matrix. These alterations lead to a gradual remodeling of the mineralized (alveolar bone) and nonmineralized (periodontium) tooth supporting tissues during the orthodontic movement [2].

Mainly during the early stage of application of orthodontic forces, an inflammatory process can occur in the periodontium as a physiological response to the tissue stress. Several potential biomarkers of the biological alterations after an orthodontic force application may be detected, specifically expressing bone resorption and formation, periodontal ligament changes, and vascular and neural responses.

The appropriate choice of the mechanical force to induce the highest rate of tooth movement in the shortest period of treatment avoiding adverse consequences is a primary objective of a specialist. To identify the degree of remodeling occurring in the periodontal tissues during an orthodontic treatment by monitoring the levels of certain biochemical mediators may be a clinically useful procedure.

In this chapter, the importance of evaluating the levels of substances as valid biomarkers of periodontal effects of an orthodontic treatment is emphasized through a description of the specific role of each of them. Only studies on mice and rats have been considered as animal models, whereas in order to monitor the expression of these biomarkers noninvasively in humans, changes in the composition of gingival crevicular fluid (GCF) during orthodontic and orthopedic tooth movement have been selected as first choice. Substances involved in bone remodeling are produced by periodontal ligament cells in sufficient quantities to diffuse into the gingival crevicular fluid. Thus, by means of two different study models "mice and men," the clinical usefulness of some biomarkers in orthodontics is properly analyzed.

## 2. Anatomy and function of periodontal ligament and alveolar bone

"Periodontium" comes from the Greek "what is around the tooth." It includes all structures that anchor the tooth to the alveolar bone surrounding forming a strong support structure [3]. Its components are gingiva, periodontal ligament, cement, and alveolar bone (**Figure 1**).

The periodontal ligament is the structure interposed between the tooth root and the alveolar bone, with an area of 0.25–0.5 mm. It is a connective tissue whose main component is represented by a set of elastic collagen fibers, parallel to each other, inserted on one side in the cementum and on the other in the lamina dura of the alveolar bone (Sharpey's fibers). The oblique direction of these supporting fibers on the tooth surface provides the tooth with enough elasticity to distribute the masticatory forces over a large surface of the alveolar process, allowing the
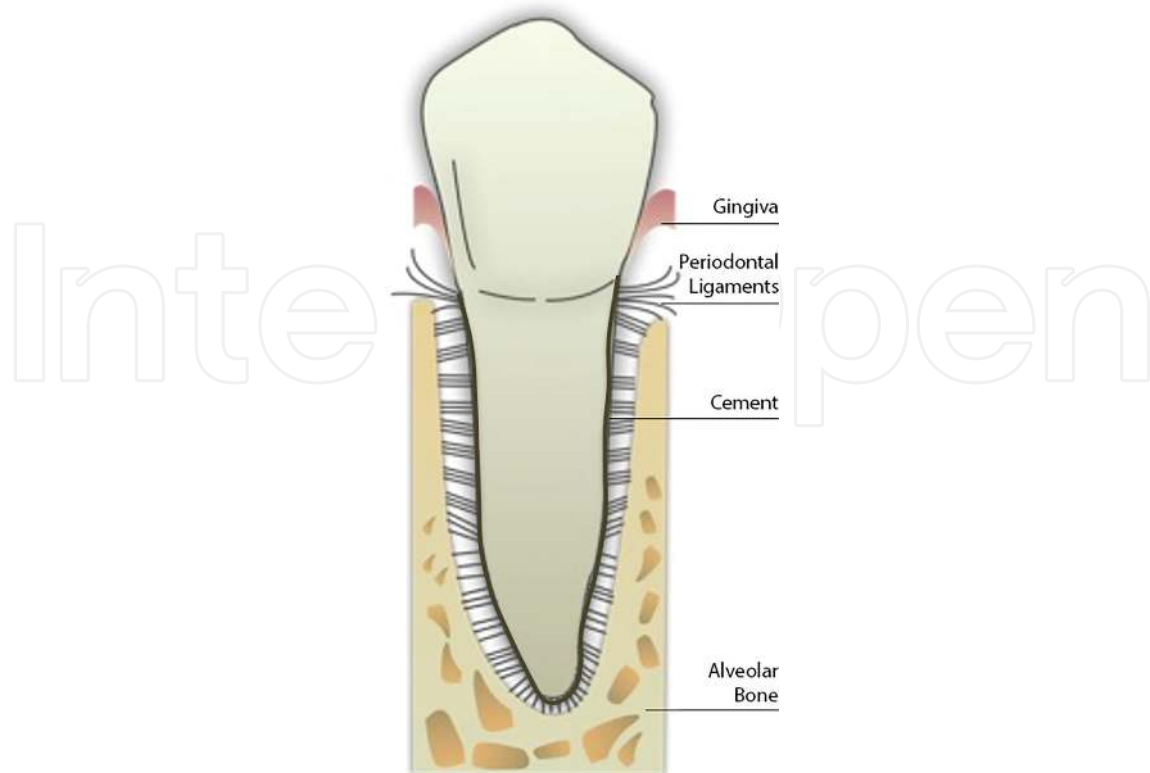
**Figure 1.** The tooth and its supporting tissues: gingiva, periodontal ligament, cement, and alveolar bone.

tooth to oppose a greater resistance to forces exerted during masticatory function. The other component of the periodontal ligament is the cellular element consisting of undifferentiated mesenchymal cells and their lines of differentiation in fibroblasts and osteoblasts, together with the neural elements, and vascular tissue fluid from the circulatory system.

The alveolar bone or cortex alveolaris is the portion of the jaws functionally dependent on the teeth. The alveolar bone forms the socket where the tooth is located. Under physiological conditions, the alveolar bone is located approximately 2 mm apical to the cement-enamel junction (CEJ). In general, the bone is a mineralized connective tissue, which consists of organic material. Bone is a very dynamic tissue remodeling continuously throughout life. Histologically, it is a particular type of connective tissue of support, consisting of cells dispersed in an abundant extracellular matrix, formed by fibers, and amorphous substance of glycoproteic origin. The bone tissue is composed of two associated phases: organic and mineral inorganic. The organic phase is constituted for 90% of collagen type I. The mineral phase is constituted by calcium and phosphorus combined in a little crystallized hydroxyapatite, and other ions which usually are found in the surface layers.

Osteoblasts and osteoclasts constitute the bone cellular components. The former synthesize and secrete the organic matrix. Osteoblastic cells, after the deposition of the matrix that is subsequently mineralized, remain embedded in it turning into osteocytes, which remain in mutual connection via cytoplasmic extensions, and also in vasal connection through a series of small channels. The osteoclasts, instead, are multinucleated cells formed by fusion of precursor cells

consisting of hematopoietic stem cells from the family of mononuclear phagocytes. They are the mediators of the continuous resorption of bone. Osteoclasts occupy small depressions on the bone's surface, called Howship's lacunae, caused by erosion of the bone by specific enzymes.

The characteristic trabecular structure is constituted by external component of the compact bone and the inner cancellous bone, providing a great resistance to mechanical stress [4].

## 3. Periodontal and bone responses to physiologic masticatory activity

Orthodontic therapy applies light and continuous forces to the teeth and to the related facial structures. The term biomechanics in orthodontics refers to the complex reactions in response to a specific orthodontic force application [5]. Each tooth is attached to the surrounding alveolar bone by the periodontal ligament (PDL), a robust structure collagen support around the tooth root with cellular components and tissue fluids. These last two elements play a key role in normal physiological masticatory system. The collagen of the ligament, the alveolar process and cementum are constantly subjected to remodeling and renovation during normal masticatory activities [6]. The fibroblasts in the periodontal ligament have similar features to osteoblasts and, standing out from the focal cell population, produce new tissue bone. Osteoclasts and cementoclasts remove alveolar bone and cementum, respectively. During the physiological process of mastication, dental and periodontal structures are subjected to both heavy and intermittent forces. In particular, the teeth are subjected to loads that vary from 1 to 2 kg when chewing soft food up to 50 kg for tough food. During heavy masticatory loads lasting maximum 1 second, the displacement of a single tooth in the periodontal space is prevented by the naturally incompressible fluid, and the loading force is transmitted to the alveolar process walls, which are consequently flexed. They generate currents stimulating bone regeneration and repair, thus allowing the adaptation of the bone architecture to the changed function.

## 4. Periodontal and bone responses to the application of orthodontic forces

Bone and periodontal responses to orthodontic treatment mainly depend on the force duration and its intensity applied to the teeth. The orthodontic biological mechanisms can be evaluated considering two different theories: bioelectrical theory and pressure-tension theory. According to the first theory, the bone subjected to bending generates piezoelectric currents that determine changes in bone metabolism. These currents are constituted by electrons (e−) moving from side to side of the network of a crystalline material; thus, the orthodontic forces routinely produce an alveolar bone deflection, and these strains lead to changes in the periodontal ligament. For the theory of the pressure tension, the cell differentiation and the subsequent tooth movement are controlled by chemical signals. A continuous force induces a compression of the ligament in some areas, with reduction of oxygen tension and then of blood flow, whereas in other areas, a traction of the ligament with increased oxygen tension and equal or increased blood flow (**Figure 2**) [5].

Modifications of the irroration are accompanied by rapid chemical changes, which can stimulate the differentiation and activation of specialized cells for bone and periodontal remodeling. Cellular destruction and injury of capillaries lead to an inflammatory reaction followed by formation of new capillaries and connective cells. In particular, the compression of the periodontal fibers causes the "hyalinization" [7] characterized by the disappearance and/or pycnosis of cell nuclei and the convergence of the collagen fibers in a gelatinous-like substance. Reitan in his papers on the histological changes following orthodontic force application [8] reported that the hyalinization refers to cell-free areas within the PDL, in which the normal tissue architecture and staining characteristics of collagen in the processed histological material have been lost. He observed that the hyalinization occurred within the PDL following the application of even minimal force. After the elimination of the hyalinized tissue, a direct resorption occurs thanks to the activation of the osteoclasts from the PDL, and then, an indirect resorption with cellular elements from the blood flow occurred. The pressure exerted on the periodontal ligament is directly proportional to the reduction of the blood flow inside the PDL until a complete collapse of the blood vessels and to a consequent ischemia. If the pressure applied to the tooth is light and lasting for 1–2 seconds, the PDL is partially compressed to the displacement of the fluids outside of the periodontal space following the displacement of the tooth in its alveolus; after 3–5 seconds, the blood vessels are passively compressed by the pressure side and dilated from the tension side; fibers and cells of the PDL appear mechanically distorted. A slight pressure maintained for a few minutes causes changes in the blood flow and variations of oxygen tension, with a simultaneous release of prostaglandins and inflammatory cytokines. After at least 4 hours, metabolic changes occur by induction of several chemical modulators, increased cyclic Adenosine Monophosphate (cAMP), and cell differentiation within the periodontal ligament. After 48 hours, the orthodontic tooth movement starts after the alveolar
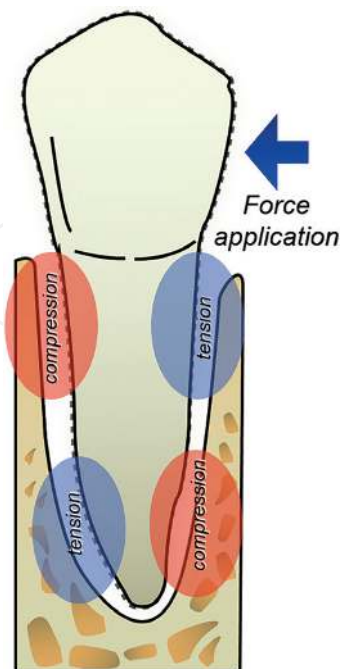


**Figure 2.** Compression and tension areas after force application.

bone remodeling that occurs through the combined activity of osteoclasts and osteoblasts. If pressure on dental structure is high, after 3–5 seconds, the blood vessels in the PDL collapse on the compression side, and after a few minutes, the interruption of blood circulation occurs in that area of PDL, with sterile necrosis and disappearance of the cellular component (areas of hyalinization). In this case, the remodeling takes place thanks to cells from contiguous areas, which begin to invade the necrosis causing an indirect resorption, since the action of osteoclasts starts from the outer part of the lamina dura. Consequently, the mechanisms of hyalinization and resorption indirectly involve an inevitable delay in the displacement of the tooth, in addition to the pain caused to the patient, due to the presence of ischemic and inflamed areas in the PDL. The ideal intensity for orthodontic forces is when it promotes tooth movement producing cell differentiation without the complete occlusion of the blood vessels in the periodontal ligament, and, therefore, the biological effect of an orthodontic force depends on its intensity and area of PDL involved, or by the pressure on the tooth.

Therefore, orthodontic tooth movement could be divided in three phases: the initial phase, the lag phase, and the postlag phase. The initial phase is characterized by immediate and rapid tooth movement and occurs from 24 to 48 hours after the first application of force. This rate is largely attributed to the displacement of the tooth in the PDL space. The lag phase lasts from 20 to 30 days and shows relatively little tooth displacement. This phase is marked by PDL hyalinization in the region of compression. No subsequent tooth movement occurs until the cells complete the removal of the main part of the necrotic tissues. The lag phase is followed by the postlag phase, during which the rate of movement increases [9].

The sequence of events following orthodontic tooth movement can be characterized using suitable biomarkers (**Figure 3**).
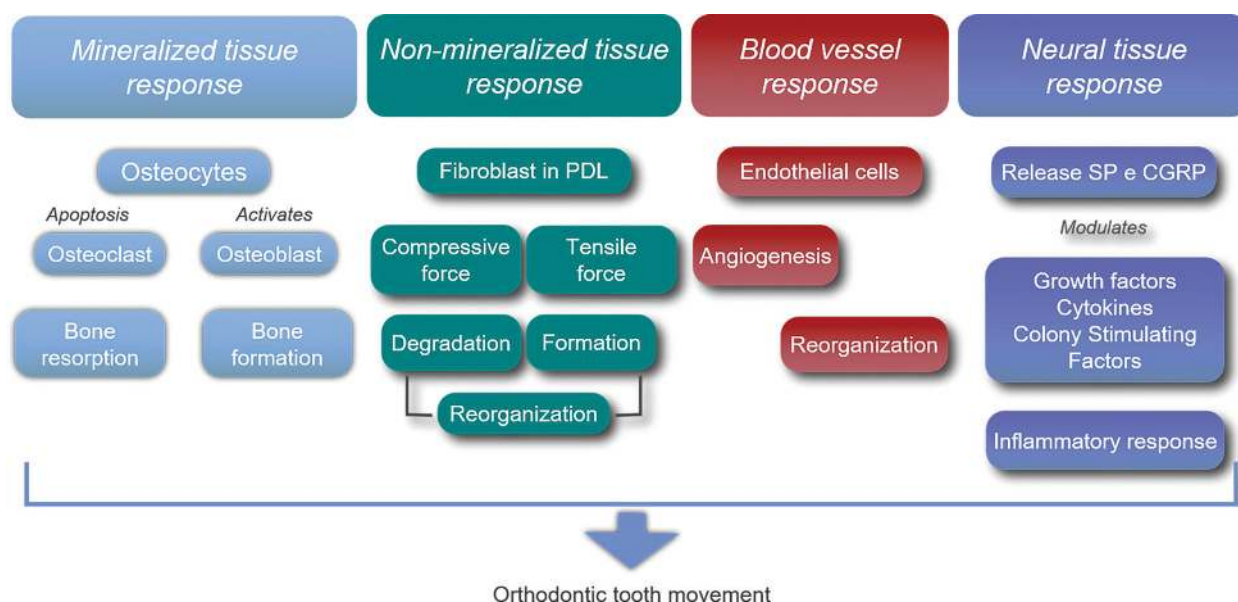


**Figure 3.** Effects of orthodontic force application on periodontal tissues.

## 5. Two different models "mice and men" to analyze the orthodontic tooth movement

### 5.1. Mice as models of study of periodontal and bone tissue remodeling after orthodontic tooth movement

Up to now, a large number of studies in various species of animals have been carried out to evaluate the biological response on the periodontal ligament after a force application. Mice are the most widely used animals to study tooth movement (**Figure 4**), even if there are advantages or disadvantages [10].

To note among the disadvantages, the alveolar bone of mice is denser than in humans, and there are no osteons. Indeed, the animal osteoid tissue along the alveolar bone surface is less, a few mucopolysaccharides are contained in the extracellular matrix, and the calcium concentration is more controlled by intestinal absorption than by the bony tissue. Some disparities have also been reported in the arrangement of the peritoneal fibers, in the supporting structures, as well as in root formations that are faster. However, mice are considered a good model to study the orthodontic tooth movement consequences. They are relatively inexpensive facilitating the use of a large sample and the housing for a long period of time; the histological preparation of their material is easier than in other animals; most antibodies required for cellular and molecular biological techniques are only available for mice and rats; moreover, transgenic strains are almost exclusively developed in small rodents. The difference between mice and rats poses greater difficulty in placing an effective orthodontic appliance in the smaller mouth of a mouse. A systematic review on the literature for experimental tooth movement in rodents showed several shortcomings in part related to the physiology of the animals and on the other hand to the design of the orthodontic appliance [11]. In consideration of this critical evaluation, the animal model was useful to achieve the current knowledge on experimental orthodontic tooth movement.

### 5.2. Evaluation of bone and periodontal tissue responses to orthodontic movement in men with the gingival crevicular fluid

The gingival crevicular fluid (GCF) is an exudate derived from epithelium lining of the gingival sulcus has been recognized for over a century. However, the exact nature of the fluid, its origins, and its composition have been the subject of controversy for decades [12, 13]. Investigations into the protein content of the GCF reported that in healthy gingival crevices, the GCF has a similar protein concentration to interstitial fluid, which was notably lower than in serum [14–16]. On the contrary, an inflamed gingiva has GCF with raised protein concentrations that are thus similar to those of serum [17]. Subsequently, upon local inflammation or injury, the GCF would become an inflammatory exudate. The increased GCF flow contributes to host defense by flushing bacterial colonies and their metabolites away from the sulcus, thus restricting their penetration into the tissue [18].

The range of the GCF constituents is very large, as it can contain both human and bacterial cells and many different molecules. Among the most representative cellular components of the GCF, there are the leukocytes, especially neutrophils, which have important roles in the
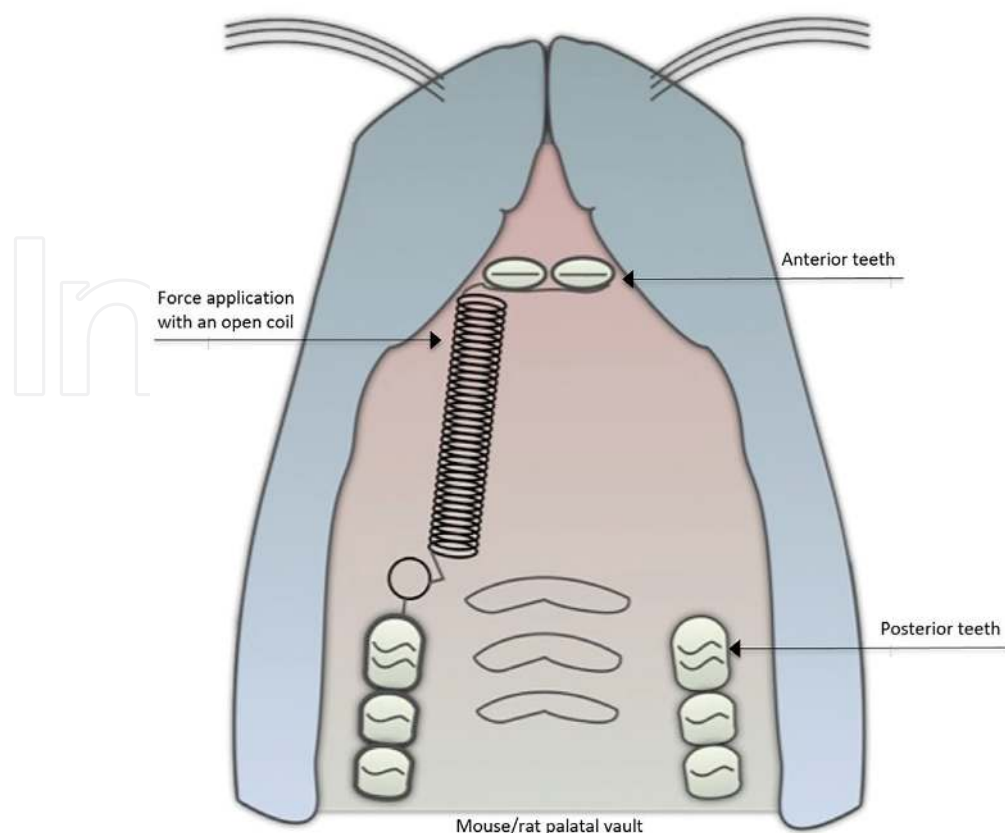
**Figure 4.** A schematic drawing of the animal model with the application of a force by an open coil between anterior and posterior teeth to induce a tooth movement.

antimicrobial defense of the periodontium [19]. However, while the bacterial and cellular components of the GCF are of primary concern for clinicians and researchers, its molecular contents represent a promising source of biomarkers in dentistry and in orthodontics for the monitoring of site-specific tissue remodeling leading to the tooth movement [20] and, as revealed more recently, for the evaluation of skeletal maturation on an individual basis [21].

The host molecular content of the GCF includes a large variety of molecules that have the potential to be classified as biomarkers of cell death, tissue damage, inflammation, bone resorption, bone deposition, and others, according to their specific biological functions. For several of these biomarkers, associations between their levels and specific clinical conditions have been shown along with the predictive value for the biomarkers; for instance, in terms of tissue destruction due to periodontitis. Several recent studies on orthodontic tooth movement have used biochemical assay analysis of GCF as a simple and noninvasive procedure for repetitive sampling from the same site [22].

### 5.2.1. Methods of collection of gingival crevicular fluid

The GCF can be collected by different methods. The most used methods in the literature are (1) the gingival washing technique; (2) the capillary tubing or micropipettes; and (3) the use of absorbent filter paper strips.

*5.2.1.1. The gingival washing technique*

This technique was described a long time ago [23]. The gingival crevice is perfused with a determined volume of an isotonic solution ejected from a microsyringe and then re-aspirated from the gingival crevice at the interdental papilla. The fluid collected represents a dilution of the GCF, and it will contain both cells and soluble constituents, such as plasma proteins. As a major disadvantage, this procedure can fail to recover all the instilled fluid or the GCF contents during the re-aspiration. Thus, an accurate quantification of the GCF volume or composition is not guaranteed as the precise dilution factor cannot be determined. This technique is particularly valuable for harvesting cells from the gingival crevice region.

*5.2.1.2. Capillary tubing or micropipettes*

This collection method was described more than 40 years ago [24]. It consists on the use of capillary tubes of specific internal diameter inserted into the gingival crevice after the isolation and drying of the area. Due to the known internal diameter of the capillary tubes, it is possible to determine the exact GCF volume collected, through the measure of the GCF migration along the capillary tubing. However, it is difficult to collect an adequate volume of GCF in a short period, unless the sites are inflamed and contain large volumes of fluid. In fact, collection times from an individual site may exceed 30 minutes, thus making the capillary holding difficult and possibly traumatic for periodontal tissues. Moreover, this can cause the release of a serum-derived fluid that may alter volume and composition of the GCF [25]. A further disadvantage of this method is the challenging removal of the full GCF sample from the capillary tubes.

*5.2.1.3. Absorbent filter paper strips*

The use of adsorbent paper strips represents the procedure most used for GCF collection today [25, 26]. In this procedure, the standardized absorbent paper strips are inserted into the gingival crevice and left *in situ* for 5–60 seconds. The advantages of the technique are that it is quick and easy to use and can be applied to individual sites, and it is the least traumatic when correctly used. The main variations are the reduced timing of sampling and the volume estimation of the collected sample. Because of this methodological variability, the data from different studies need to be interpreted with caution, considering how exactly the collection of the GCF was performed with the paper strips. The methods of collection may be broadly divided into the intracrevicular and extracrevicular techniques. The former depends on the strip inserted at least 3 mm into the gingival crevice or into a periodontal pocket [27], whereas in the latter, the strips are inserted until the "minimum resistance" is felt [28] in an attempt to minimize trauma. A problem with GCF collection and data interpretation may be the sample contamination by blood, saliva, or bacterial plaque. A careful isolation should be performed to minimize the potential GCF contamination. Before performing any biochemical analysis, the volume of the GCF sample must be determined. To achieve the recovery of strips, it is necessary to separate the GCF from the filter paper strips, and protein recovery is close to 100% using a centrifugal elution technique [29].

# 6. Biomarkers of periodontal and bone responses to orthodontic force application

Orthodontic tooth movement induces a series of orchestrated cellular and molecular events responsible for connective tissue remodeling and osteoclast activation [30].

Thus, the sequence of cascades following the mechanical stress from orthodontic appliances can be characterized using suitable biomarkers.

## 6.1. Pro-inflammatory cytokines: interleukin-1 (IL-1$\beta$), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-(TNF-$\alpha$), and prostaglandins E (PGE1-PGE2)

IL-1$\beta$ is one of the most abundant cytokines in the periodontium during the initial stage of orthodontic tooth movement [3]. In the early stages of tooth movement (at 12 and 24 hours), the following cells stained positively for IL-1$\beta$: fibroblasts, macrophages, cementoblasts, cementoclasts, osteoblasts, and osteoclasts [31, 32]. IL-1$\beta$ seems to be primarily secreted by macrophages, whereas macrophage accumulation in compressed periodontal areas has been detected at later stages of the treatment. Thus, during the initial stage of tooth movement, IL-1$\beta$ derives from other periodontal cell types, like the osteoclasts, as immediate response to mechanical stress.

The experimental tooth movement leads to significantly increased recruitment of cells belonging to the mononuclear phagocytic system. It was suggested that the neuroimmune interactions may be of primary importance in the initial inflammatory response, as well as the regenerative processes of the periodontal ligaments are incident to orthodontic tooth movement [33]. IL-1$\beta$ is involved in the survival, fusion, and activation of osteoclasts. This role is significant since the rate of tooth movement correlates with the quantity of bone remodeling in the alveolar process [34]. Higher levels of expression of inflammatory cytokines and their related receptors have been shown after an inflammatory process induced by perforating the buccal cortical plate of orthodontically treated rats. The concentration of the IL-1$\beta$ mRNA in the rats' periodontal ligament is increased within 3 hours after orthodontic force loading, mainly on the pressure side [35, 36].

The particular function as pro-inflammatory cytokine of IL-1$\beta$ has been demonstrated by the administration of exogenous IL-1 receptor antagonist (IL-1RA) [37]. IL-1RA treated mice showed a 66% decrease in the levels of IL-1$\beta$ when compared to the experimental tooth movement of vehicle treated mice, and this was associated with a reduction of the number of osteoclasts in the pressure side of periodontal tissues after histological characterization. Therefore, IL-1RA down-regulates orthodontic tooth movement because of the lower rate of tooth displacement in mice treated with IL-RA therapy. IL-1$\beta$ is also considered a potent inducer of IL-6 production: it overlaps with IL-6 and TNF-$\alpha$ in their actions [38]. IL-6 regulates immune responses in inflammation sites [39], and it has an autocrine/paracrine activity stimulating osteoclast formation and the bone-resorbing activity of preformed osteoclasts [40]. IL-6 production increases after 24 hours [41].

Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) is another pro-inflammatory cytokine shown to elicit acute or chronic inflammation and stimulate bone resorption [32]. TNF-$\alpha$ directly stimulates the differentiation of osteoclast progenitors to osteoclasts in concert with the macrophage-colony stimulating factor (M-CSF). Tuncer et al. [42] reported the increased levels of IL-8 at PDL tension sites and proposed it as triggering factor for bone remodeling.

Many studies of the gingival crevicular fluid have confirmed the increased levels of these pro-inflammatory cytokines during periodontal tissue remodeling after orthodontic tooth movement [41, 43, 44].

Other clinical and animal investigations have also identified a primary role of prostaglandins E (PGE1 and PGE2) in stimulating bone resorption [45, 46]. Prostaglandins are produced from the arachidonic acid, which in turn derives from phospholipids. The liberation of prostaglandins constitutes the first response to the pressure stimulus; it occurs when cells are mechanically deformed, and the consequent mobilization of membrane phospholipids leads to the formation of inositol phosphate (IP), an important chemical messenger. PGE2, in particular, is able to mediate inflammatory responses and induce bone resorption by osteoclastic cell activation [9]. The literature reports that prostaglandins directly stimulate osteoclast production and their capacity to form ruffled border and effect bone resorption. In addition, the PGE2 level in GCF reflects the biologic activity in the periodontium during orthodontic tooth movement, and it is significantly increased in both tension and compression sides [47].

### 6.2. RANK/RANKL/osteoprotegerin (OPG) system

During orthodontic movement, a variety of proliferation markers are expressed: KI-67 and receptor activator of nuclear factor-Kappa β ligand (RANKL) [48, 49] indicate the recruitment of osteoclasts in compression areas, whereas Runx2 [50], Col1-GFP, and BSP-GFP expression cells express the increase of differentiated osteoblasts in tension areas [51]. To note, the TNF-related ligand, the receptor activator of nuclear factor-Kappa β ligand (RANKL), and its decoy receptor RANK, as well as the osteoprotegerin (OPG), were found to play important roles in the regulation of bone metabolism. RANKL is a downstream regulator of osteoclast formation and activation, through which many hormones and cytokines produce their bone resorption effect. In the bone tissue, RANKL is expressed on osteoblast cell lineage and exerts its effect by binding the RANK receptor on osteoclast lineage cells. This binding leads to rapid differentiation of hematopoietic osteoclast precursors to mature osteoclasts. OPG is a decoy receptor produced by osteoblastic cells in competition with RANK for RANKL binding. The biological effects of OPG on bone cells include inhibition of terminal stages of osteoclast differentiation, suppression of activation of matrix osteoclasts, and induction of apoptosis. Thus, bone remodeling is controlled by a balance between RANK-RANKL binding and OPG production. Kanzaki et al. [52] reported that OPG gene inhibited RANKL-mediated osteoclastogenesis and experimental tooth movement in rats. Thus, the inhibition of the activity of RANKL in its promoting osteoclast differentiation could be very helpful in preventing, for instance, tooth anchorage during orthodontic treatment and relapse during the posttreatment period.

### 6.3. Macrophages-colony-stimulating factors (M-CSFs)

Colony-stimulating factors (CSFs) comprise those related to granulocytes (G-CSFs), macrophages (M-CSFs), or to both cell types (GM-CSFs). They have a great implication in bone remodeling through osteoclast formation and thereby during tooth movement [9]. These molecules are specific glycoproteins interacting to regulate production, maturation, and function of granulocytes and monocyte macrophages. Therefore, M-CSF plays an important role during the early osteoclast differentiation, which increases the rate of osteoclastic recruitment and differentiation during initial phases of orthodontic tooth movement [50]. In particular, optimal dosages of M-CSF are correlated with measurable changes in tooth movement and gene expression, providing potential for clinical studies in accelerating tooth movement.

### 6.4. Vascular endothelial growth factor (VEGF) as a key factor of neovascularization

Vascular endothelial growth factor (VEGF) is the primary mediator of angiogenesis and increases vascular permeability during tissue neoformation, always associated to the presence of blood vessels [53]. During orthodontic tooth movement, compressive forces induce angiogenesis of periodontal ligaments and the activation of the vascular endothelial growth factor [54]. A study performed the localization of VEGF *in vivo* in the periodontal tissues of 15 male Wistar rat during an experimental tooth movement. A compressive force at 150 mN was applied by a standardized compressive spring placed between the right and left upper first molars in each rat's mouth. The maxillary bone was analyzed with immunohistochemical staining. VEGF immunoreactivity was in vascular endothelial cells, osteoblasts, osteoclasts in resorption lacunae, in fibroblasts adjacent to hyalinized tissue, a local necrotic area in compressed zone, and in mononuclear cells in periodontal tissues from animals. VEGF mRNA was also found in fibroblasts and osteoblasts in tension area of mice periodontal ligament during experimental tooth orthodontic movement [55]. The protocol included 10 mice, divided between experimental and control animals, and provided the assessment of premaxillary bone frontal sections [56].

Therefore, VEGF has a relevant role in remodeling periodontal ligament as well as in bone resorption and formation.

### 6.5. Neuropeptides during neural tissue response to orthodontic tooth movement

During orthodontic tooth movement, a neurogenic inflammation occurs in the periodontium with an increased concentration of specific proteins. Somatosensory neurons disseminate signals from periodontal peripheral nerve fibers to the central nervous system. With application of physiologic orthodontic force, periodontal peripheral nerve fibers release calcitonin gene-related peptide (CGRP) and substance P, acting as neurotransmitters. Moreover, CGRP and substance P are vasodilators, inducers of increased vascular flow and permeability (diapedesis), and stimulators of plasma extravasation and leukocyte migration into tissues (transmigration).

CGRP activates the bone formation through osteoblast proliferation and osteoclast inhibition. Receptors for CGRP are revealed on osteoblasts, monocytes, lymphocytes, and mast cells. Receptor activation results in amplified intercellular communication, promoting cytokine (inflammatory mediator molecules) synthesis and release.

Healthy periodontal and alveolar bone innervation promotes maximum blood flow during orthodontic tooth movement, whereas denervation reduces blood flow and bone formation [57]. Substance P (SP) is another sensory neuropeptide released from the peripheral endings of sensory nerves. It can modify the secretion of pro-inflammatory cytokines from immunocompetent cells during periodontal tissue remodeling. Worthy of note, SP stimulated the production of PGE2 [58].

### 6.6. Enzymes reflecting biological activity in periodontium: caspase-1, β-glucuronidase (β-G), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)

In conjunction with the inflammatory process, there is an apoptotic process occurring to eliminate the hyalinized periodontal tissue formed during the early stages of orthodontic movement. Caspase-1 is the most important mediator of inflammation and apoptotic responses, activated by inflammatory signals as alterations in the intracellular ionic milieu. It has the role to process and activate pro-IL-1β and other pro-inflammatory cytokines. In a rat model under orthodontic treatment, caspase-1 mRNA expression is increased, and the level of caspase-1 changes with different temporal phases of orthodontic tooth movement [59]. An irreversible root resorption and deformation of periodontal tissues might emerge: an excessive local orthodontic force application or in some diseases like rheumatoid arthritis related to the hyperexpression of caspase-1. In these cases, a method to preserve the structure of periodontal ligaments may be the administration of the inhibitors of caspase-1 activity such as VX-765 [60] and Pralnacasan [61]. A biomarker of primary granule release from polymorphonuclear leukocytes is the lysosomal enzyme β-glucuronidase (βG). Increased levels of this enzyme have been found in the GCF of adolescents treated with rapid maxillary expander, thus during orthodontic and orthopedic movement. Moreover, βG, similar to other biochemical mediators as IL-1β, correlates to both direct and indirect application of mechanical stimuli, with an increased level that is higher following stronger forces [62].

Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are soluble enzymes usually confined to the cytoplasm of cells but released to the extracellular environment upon cell necrosis. The AST and LDH activities into the GCF have also been assessed during orthodontic treatment. The GCF AST activity is significantly increased in both the tension and compression sites at days 7 and 14. This amount is explained as a consequence of a controlled trauma that leads to an increased cell necrosis after the mechanical stress on the periodontal ligaments and alveolar bone. A low increase of GCF AST activity reflects the application of orthodontic force on teeth, particularly on compression side, while an occlusal trauma leads to a higher amount of enzymatic level [63]. The enzyme lactate dehydrogenase, likewise, is normally limited to cytoplasm, and it is only released extracellularly after cell death and tissue breakdown [64]. This enzymatic activity is greater in compression sites because of the typical process that occurs during periodontal remodeling resulting from orthodontic tooth movement: there is an early wave of resorption of 3–5 days followed by its reverse process of 5–7 days, and a late wave of formation that lasts for 7–14 days, on both the pressure and the tension sides of the alveolar wall.

## 6.7. Enzymes involved in bone cell activities: alkaline phosphatase (ALP) and acid phosphatase (ACP)

The biological alteration due to the orthodontic tooth movement involves alterations above all in the surrounding bone tissue [65]. Bone metabolism is associated with alkaline phosphatase (ALP) and acid phosphatase (ACP) expressed by osteoblasts and osteoclasts, respectively. ALP is a ubiquitous tetrameric enzyme associated with the plasma membrane of cells, also found in liver, intestine, and placenta, and it is observed during healing of bone fractures and physiologic bone growth. The bone isoenzyme predominates in childhood and particularly during puberty [66]. These are 507 amino acid proteins encoded by the same gene but differ in their degree of glycosylation. The enzymes catalyze the hydrolysis of monoesters of phosphoric acid and a transphosphorylation reaction with large concentrations of phosphate acceptors [67]. Alkaline and acid phosphatases are released by injured, damaged, or dead cells into extracellular tissue fluid, and, in general, high enzyme activity is an expression of greater cellular activity. ALP activity is found at much higher levels in the periodontal ligament than in other connective tissues [68]. After an orthodontic force application, these enzymes are produced in the periodontium and diffuse in the site-specific GCF. Thus, the monitoring of phosphatase activities in the GCF could be suggestive of the tissue changes occurring during orthodontic tooth movement. In fact, experimental studies in rats and clinical studies in humans correlate alveolar bone remodeling with changes in GCF phosphatase activities [26, 69–72]. To identify and understand the enzymatic changes occurring during the early stages of orthodontic tooth movement and to coincide with initial and lag phases of tooth movement, the studies consider an orthodontic cycle of duration 21 days. It was observed that the ALP activity peaked on the 14th day in most patients, followed by a sharp fall by the 21st day. The activity decrease is related to removal of the hyalinized zone. When the enzyme activity is high, the tooth movement rate is greater. This implies that the ALP activity follows the rate of tooth movement during the initial phases. In the hard bony tissues, the ALP has been implicated in the process of mineralization. Active osteoblasts and osteocytes give an intense staining reaction for alkaline phosphatase. No enzyme activity is found in bone matrix, except when it is in close association with matrix-synthesizing cells. The osteogenic cells in the periodontal ligament react to the tensional forces with an increase in the maturation level. The fibroblast proliferation and collagen have been shown to increase in the tension sites. The ALP activity is lower in the compressed hyalinized zones of the periodontal ligament, whereas ACP activity is higher. After 7–14 days of orthodontic force application, the bone deposition occurs in both tension and pressure sites of the alveolar wall. The main bone remodeling activity at the early times in a remodeling cycle is resorptive, but in the later phase, resorption and deposition become synchronous. This might be due to increased acid phosphatase activity that has been observed in the early phases of orthodontic tooth movement. High levels of alkaline phosphatase have been described after 7 days, when bone deposition begins, and a significant peak occurs on day 14. It is obvious that, as a forerunner to bone formation, the number of fibroblasts and osteoblasts increases in areas of tension. This occurs as a result of increase in cell number by mitotic cell division. The histologic studies showed that in marginal tensional areas, cell proliferation occurs between 36 and 50 hours and lasts for 10–21 days. The tension causes shape changes, and osteoblasts move slightly apart. On the compression side, bone resorption would occur, and osteoclastic activity would be high with little or no osteoblastic activity.

To note, ALP activity is influenced by clinically detectable dental displacements and also by mechanical stress and gingival inflammation.

In conclusion, the analysis of the ALP associated with bone metabolism, under healthy gingival conditions, is a suggestive indicator of the histological and biochemical changes in bone turnover and therefore of the rate/amount of tooth movement. Moreover, the properties of the GCF ALP activity distinguishing between clinically moving and nonmoving teeth show that this enzyme should be further studied as a diagnostic tool in orthodontics [22].

## 7. Conclusions and clinical relevance

When exposed to different degrees of magnitude, frequency, and duration of mechanical loading, alveolar bone and periodontal ligaments show extensive macroscopic and microscopic changes. Force application on the tooth also alters periodontal tissue vascularity and blood flow, resulting in the local synthesis and release of various molecules, such as cytokines, growth factors, colony-stimulating factors, enzymes, and neurotransmitters [73].

A biomarker is a substance that can be objectively measured revealing any process occurring during a therapeutic treatment. The several potential biomarkers of the biological alterations after an orthodontic force application described in this chapter may be significantly useful to perform an appropriate choice of the mechanical force to achieve the right rate of tooth movement and to accelerate the orthodontic time, avoiding adverse effects such as root resorption or bone loss [74–76].

Finally, a clinical use of these biomarkers for the specialists may be mandatory to improve orthodontic therapies.

The different experimental and clinical methods for the collection and assessment of these potential biological markers were described in both animal and human models. GCF analysis, especially, offered several advantages for its simple, quick, and noninvasive collection.

Overall, a detailed knowledge of the ongoing process occurring in periodontal tissues during orthodontic procedures can lead to proper choice of mechanical loading with the aim of shortening the period of treatment and avoiding adverse consequences associated with orthodontic treatment.

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