Inflammatory side effects associated with orthodontic tooth movement

GIANNOPOLOU, Catherine

Abstract

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by the remodeling of the periodontal ligament and alveolar bone. The remodeling activities and the ultimately tooth displacement are the consequence of an inflammatory process. Vascular and cellular changes were the first events to be recognized and described. With the advancement of research techniques an important number of inflammatory mediators, growth factors, neuropeptides and metabolites of arachidonic acid have been detected in the surrounding periodontal tissue. Most of them are produced in sufficient amounts to diffuse into the gingival crevicular fluid and their analysis has been used to evaluate the local cellular metabolism that accompanies bone remodeling process during orthodontic tooth movement. Gingival inflammation, discomfort or pain and root resorption are common undesirable side effects of orthodontic treatment. Gingival health is compromised during orthodontic treatment because of alterations in the composition of bacterial plaque and consequently the development of gingivitis, especially when fixed orthodontic appliances are [...]
INFLAMMATORY SIDE EFFECTS ASSOCIATED WITH ORTHODONTIC TOOTH MOVEMENT

Thèse d'habilitation au titre de Privat-Docent à la Faculté de Médecine de Genève

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WITH ORTHODONTIC TOOTH MOVEMENT

To the memory of Professor Giorgio Cimasoni
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<tr>
<td>ALPase</td>
<td>alkaline phosphatase</td>
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<tr>
<td>ASP</td>
<td>aspartate aminotransferase</td>
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<tr>
<td>βG</td>
<td>beta glucuronidase</td>
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<td>BOP</td>
<td>bleeding on probing</td>
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<td>CBCT</td>
<td>cone-beam computed tomography</td>
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<td>DPP</td>
<td>non-collagenous proteins of dentin matrix</td>
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<td>DSP</td>
<td>dentine sialoprotein</td>
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<td>EARR</td>
<td>external apical root resorption</td>
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<td>EGF</td>
<td>epidermal growth factor</td>
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<td>GCF</td>
<td>gingival crevicular fluid</td>
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<td>GI</td>
<td>gingival index</td>
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<td>IL-1β</td>
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<td>LDH</td>
<td>lactate dehydrogenase</td>
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<td>micro-CT</td>
<td>micro-computed tomography</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteinases</td>
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<tr>
<td>NSAIDS</td>
<td>nonsteroid anti-inflammatory drugs</td>
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<td>OC</td>
<td>osteocalcin</td>
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<tr>
<td>OPG</td>
<td>osteoprotegerin</td>
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<td>osteopontin</td>
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<tr>
<td>OPT</td>
<td>orthopantomograph</td>
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<tr>
<td>PDL</td>
<td>periodontal ligament</td>
</tr>
<tr>
<td>PGE1</td>
<td>prostaglandin E1</td>
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<tr>
<td>PGE2</td>
<td>prostaglandin E2</td>
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<tr>
<td>PI</td>
<td>plaque index</td>
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<td>PPD</td>
<td>periodontal probing depth</td>
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<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
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<td>RANK</td>
<td>receptor activator of nuclear factor-kappa</td>
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<td>Abbreviation</td>
<td>Full Name</td>
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<tr>
<td>RANKL</td>
<td>receptor activator of nuclear factor-kappa ligand</td>
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<td>SP</td>
<td>substance P</td>
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<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<tr>
<td>TNFα</td>
<td>tumor necrosis factor alpha</td>
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<tr>
<td>VAS</td>
<td>visual analogue scale</td>
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<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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INTRODUCTION

*Inflammation* has been defined as a response of body tissues to injury or irritation, characterized by pain, swelling, redness and heat. *Orthodontic tooth movement* has been defined as the result of a biologic response to interference in the physiologic equilibrium of the dentofacial complex by an externally applied force (1). Only small amounts of force – 2 to 150g per tooth – might be required to effect this outcome which is accompanied by remodeling changes in dental and paradental tissues, including the dental pulp, the periodontal ligament (PDL), the alveolar bone and the gingival. A pre-condition for the remodeling activities, and ultimately tooth displacement, is the occurrence of an inflammatory process. Orthodontic forces along with increased vascular permeability and cellular infiltration, trigger inflammatory process in the involved dental and paradental tissues. Lymphocytes, monocytes and macrophages invade these tissues, enhancing the synthesis and release of various neurotransmitters, cytokines, growth factors, and metabolites of arachidonic acid, thus leading to an increased osteoclastic activity (2). Orthodontics is probably the only speciality that uses the inflammatory process as a means of solving functional or aesthetic problems as no tooth movement can be achieved without inflammation. However, orthodontically induced inflammation is an aseptic inflammation, does not represent a pathological condition and the expression of inflammatory mediators is transitory.

2.1. Tissue reaction during orthodontic tooth movement

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament and alveolar bone. The remodeling activities and ultimately the tooth displacement are the consequence of an inflammatory process. Prolonged application of mechanical forces that exceed the bio-elastic limits of tooth supporting structures, represents a physical agent capable of inducing an inflammatory reaction in the connective dental tissues leading to adaptive proliferation and remodeling, mainly in the PDL and alveolar bone (3-7). Over-compression results in ischaemia, interruption of nutrition and cell death with almost
unavoidable formation of a necrotic or hyalinized zone, which temporarily arrests tooth movement (4). The vascular changes are not confined only to the PDL, but encompass also the alveolar bone (8, 9), whereby proliferation of blood vessels and blood-born cells resorb the bone tissue behind the hyalinized area. The necrotic periodontal structures are potent inflammatory stimuli leading to blood vessels and cell proliferation in the surrounding areas, with removal of the hyalinized structures and subsequent tissue repair (10-12). During bone formation, proliferation of cells seems to have smaller role compared to a marked increase in differentiation of individual cells (13).

Several theories explaining alveolar tissue reactions related to tooth movement have been proposed in the past, mainly the “pressure-tension” theory (14) and the theory of the distortion or bending of the alveolar bone (15). Recently, Melsen (16) proposed that bone apposition is induced by: a) the load exerted by stretched fibers of the PDL, which will consequently induce a slight bending of the alveolar bone b) direct resorption by unloading of the alveolar wall in case of low forces and c) indirect resorption as repair due to ischemia after application of high forces.

![Figure 1: Compressed PDL after several days. When the PDL is compressed to the point that blood flow is totally cut off, differentiation of osteoclasts within the PDL space is not possible. After a delay of several days, osteoclasts within adjacent marrow spaces attack the underside lamina dura in the process called undermining resorption.](image1)

![Figure 2: On the side away from the direction of tooth movement, the PDL space is enlarged and blood vessels dilate. Expanded vessels that are only partially filled can be seen on the tension side of the PDL.](image2)

Tooth movement takes place in four phases (17, 18).
The **first phase** lasts 24h to 2 days and represents the initial movement of the tooth inside its bony socket. Cellular and tissue reactions start immediately after force application. The fibers and cells within the PDL are compressed in one side and stretched in the other, thus creating areas of “compression” and “tension”. Recruitment of osteoclast and osteoblast progenitors, as well as extravasation and chemoattraction of inflammatory cells begins. Hyalinized zones in the pressure area are present. This initial phase of orthodontic tooth movement is a typical acute inflammatory process, mainly exudative, with plasma and leukocytes leaving the capillaries and synthesis and release of various neurotransmitters, cytokines, growth factors and metabolites that act as second messengers on signal transduction pathways (e.g. arachidonic acid and prostaglandins).

The **second phase** lasts 20 to 30 days. No tooth movement but formation and removal of necrotic tissue takes place. This phase is a chronic process replacing the acute inflammatory reaction and involves various cells, such as fibroblasts, endothelial cells, osteoblasts and alveolar bone marrow cells. In the areas of compression, the PDL fibers have a distorted appearance and recruitment of phagocytic cells to remove necrotic tissues from compressed PDL sites and adjacent alveolar bone, takes place. In the areas of tension, osteoblasts are enlarged and start producing new bone matrix. New osteoblast progenitors are recruited from the population of fibroblast-like cells around PDL capillaries. Pre-osteoblasts proliferate and migrate toward the alveolar bone surface, along the stretched Sharpey’s fibers. Simultaneously, PDL fibroblasts start to multiply and remodel the surrounding matrix.

The **third phase** starts about 40 days after the initial force application, the necrotic tissue is removed and tooth movement is accelerated. This phase corresponds to the moment when the orthodontist will activate the appliance, resulting in an acute inflammation which superimposes the ongoing inflammation of the previous phase. A number of signalling molecules, such as prostaglandins, growth factors, cytokines, extracellular matrix proteins and neuropeptides are released during this phase.
In the **forth phase**, tooth movement is accelerated until total displacement.
The events taking place during the **third** and **forth phase** are similar: the collagen fibers in the pressure sides of the teeth show no proper orientation and irregular bone
surfaces, indicative of direct or frontal resorption are found. Development and removal of necrotic tissue continues to take place. As supported by Melsen (19), “indirect bone resorption at the pressure side in not a reaction of force but an attempt to remove ischemic bone lying adjacent to the hyalinized tissue. The subsequent direct bone resorption could be considered as part of the remodeling process”. In the tension sides during the third and forth phase, bone deposition takes place. It has been proposed that teeth subjected to high forces show hyalinization zones more often than teeth subjected to light forces (18, 20). However, the rate of tooth movement was found to be independent to the presence of hyalinization zones, suggesting that once tooth movement has started during the third phase, bone remodeling will take place independently of the force magnitude.

The sequence of cellular, molecular and tissue-reaction events taking place during orthodontic tooth movement has been outlined by Krishnan and Davidovitch (21).

**Figure 3**: In this hypothetical model, PDL cells under compressive strain synthesize IL-1 and IL-6 which act in an autocrine and paracrine manner to up-regulate RANKL and MMP expression by PDL cells and osteoblasts. Osteoblast-derived MMPs degrade the non-mineralized surface osteoid layer of bone, while MMPs produced by PDL cells degrade extracellular matrix. RANKL stimulates the formation and function of osteoclasts from mononuclear precursor cells which access the bone surface and degrade the mineralized matrix; deformation of the alveolar bone up-regulates MMP expression by osteocytes adjacent to the bone surface. Adapted from Meikle (2006) (22).

Remodeling of the periodontium on the tension side.

Orthodontic tooth movement can be modified by factors influencing bone metabolism (23) and by surgical accelerated procedures (24-26). Injection of prostaglandine E1 into the gingiva of moving teeth in rats and in human subjects resulted to rapid movement (27, 28). Systemic application of misoprostol, a PGE1 analog (29), of PGE2 (30) of indomethacin (31) as well as local applications of vitamin D (32), have increased significantly the velocity of tooth movement. Local and chronic application of parathyroid hormone accelerated tooth movement in rats (33). Furthermore, Verna et al (34) using a rat model, demonstrated that the rate and the type of orthodontic movement is influenced by bone turnover: in their study, bone turnover was modified pharmacologically, by inducing either hypothyroidism or hyperthyroidism in rats undergoing maxillary molar mesial movement. A systematic review on the medication effects on the rate of orthodontic tooth movement has been recently published (35).

Finally, the concept of an “optimal orthodontic force” has been the subject of investigations for several years. The concept of “light” vs “heavy” forces, of “continuous” vs “intermittent”, as well as “constant” vs “declining” has been
extensively studied. What is now accepted is that optimal force is the one capable of producing a maximal rate of tooth movement, with no tissue damage, and with maximum patient comfort (1, 36), thus optimal force may differ for each tooth and for each patient.

2.2. Side effects of orthodontic tooth movement

Inflammation is the most significant process in force-induced tissue remodeling and tooth movement. The magnitude, direction and duration of force will determine the nature of the inflammatory changes that might occur during the orthodontic treatment. However, abnormal force application and poor oral hygiene can lead to damage in teeth and periodontal tissues: all components of the craniofacial complex may be affected, such as the dental enamel, pulp, cementum, gingival, periodontal ligament (PDL), bone, cartilage and muscle. Travess et al (37) evaluated the different areas of concern and divided the risks in 3 categories: intra-oral, extra-oral and systemic. The intra-oral effects may affect: a) the gingival and periodontal ligament with the presence of gingivitis, periodontitis, gingival recession, dark triangles and poor gingival contours b) the alveolar bone with crestal bone resorption and abnormal development c) the dental roots with root resorption and early closure of the apex d) the enamel, with the presence of demineralisation areas leading to caries, fracture and excessive wear e) the pulp with pulpitis and internal root resorption and f) the soft tissues of the buccal and labial mucosa, with presence of ulcerations and trauma due to the appliances.

The extra-oral effects may affect either the face, mainly because of allergy, burns and trauma, or the tempomandibular joint with dysfunction and condylar resorption. The systemic risks include patients at risk of endocarditis who should be treated in consultation with the cardiologist. Depression, teasing and abnormal patient/parent behaviour are some psychological effects of orthodontic treatment. Recently, Krishnan (38) emphasized the importance of establishing proper communication between the patient and the orthodontist, to promote trust and improved oral hygiene habits. Whenever iatrogenic damage is observed, the patient should be immediately referred to the specialist in order to avoid exacerbating problems.
2.2.1. Gingival inflammation

The gingiva is the only dental tissue visually accessible for direct clinical evaluation of the classic signs of inflammation. During fixed orthodontic therapy, redness and swelling are commonly observed. Plaque retention associated to the orthodontic devices, causes bacterial invasion and consequently, inflammation (39). Zachrisson and Zachrisson (40) reported that, even after maintaining seemingly excellent oral hygiene, patients usually experience mild to moderate gingivitis within 1-2 months of fixed appliance placement with bands. The clinical indices, such as the Gingival Index and the periodontal pocket depth, confirm the development of an inflammatory reaction (41, 42). Unlike bone and PDL, the gingival tissue is not resorbed after orthodontic treatment, but is compressed and consequently retracted. The fact that orthodontic force does not induce gingival resorption, prevents the formation of periodontal pockets and the detachment of the tooth from the gingiva. According to a review outlining the various changes in the gingiva in response to orthodontic force application (43), disparate processes occur: first, there is an injury of the gingival connective tissue manifested by torn and ripped collagen fibers; second, the genes for both collagen and elastin are activated, whereas those for tissue collagenases are inhibited. Anti-apoptotic and proliferative stimuli are delivered to human gingival fibroblasts by mechanical strain (44). Furthermore, orthodontic treatment produces a local change in the oral ecosystem, with changes in the composition of bacterial plaque and consequently the development of gingivitis (45-48). In general, the average incidence of gingival enlargement may be four times greater around posterior teeth compared with incisors and canines (49). These differences were attributed mainly to the mechanical irritation by the orthodontic bands which are more likely to come in contact with the gingiva around the posterior than the anterior teeth, to the greater food impaction in the posterior areas and to the tendency for more effective and thorough brushing of the anterior than the posterior teeth.
2.2.2. Pain

Discomfort or pain, is a very common and almost inevitable side effect of orthodontic treatment (50). Surveys regarding the percentage of patients with pain experience during orthodontic treatment, have reported values ranging from 70% for the Caucasian population to 95% for the Asian population. Almost 91% of patients who had completed fixed orthodontic treatment, experienced discomfort or pain (51-54). It is clear that all orthodontic procedures such as placement of separators, archwire placement and activations, application of orthopaedic forces and debonding procedures result pain for the patients. It has been reported that fixed orthodontic appliances may cause more pain than removable appliances (55, 56). After appliance placement, patients report feelings of pressure, tension soreness of the teeth and pain as such. Pain begins few hours after the application of the orthodontic force and may last 5 days (52, 53, 57-59).

Being a subjective response, large variations are reported depending on several factors, such as: age, gender, individual pain threshold, emotional state and stress as well as previous pain experiences (52, 53, 54, 60). However, conflicting results have been reported on the role of each of these factors on the intensity of pain experienced by the patient. Some authors found no difference between males and females in reporting the feeling of pain, whereas others have reported that females are more “fragile” and sensitive to pain as compared to males (59-61). Conflicting results exist also on the effect of age on pain perception: some studies report that adult subjects perceive more discomfort or pain than young subjects (53) other studies report that there is no significant difference in pain perception between adolescents and adults (59) and some other studies report that adolescents experienced higher level of pain as compared to pre-adolescents and adults (62). Probably, the most significant factor in the intensity of discomfort or pain experienced by the patient after the application of orthodontic appliance, is the individual’s “physiological and psychological susceptibility” (56, 62).

The origin of pain during orthodontic treatment is thought to be in the periodontal ligament by the process of pressure, ischemia, inflammation and edema (57). It is clear that all orthodontic procedures create tension and compression zones in the PDL space. The painful experience results partly from stretching and distortion of tissues due to mechanical force, and from the interaction between inflammatory
mediators and local pain receptors. The release of various substances, such as substance P, histamine, enkephalin, dopamine, serotonin, glysin, glutamate, gamma-amino butyric acid, PGE2, leukotriens and several cytokines, has been associated to hyperalgaesia response after force application (63-72).

The association between force magnitude and intensity of pain has been a matter of debate for many years. Some authors have reported that the application of heavy force increases the rate of biological response, by causing greater periodontal compression, thus more pain (73). However, other authors found no relationship between initial tooth position, applied force levels and experienced pain (74). Presently, the main methods used to reduce the intensity of orthodontic pain, are the NSAIDS and the anesthetic gels (75).

2.2.3. Root resorption

External apical root resorption (EARR) is a common, undesirable side effect of orthodontic treatment (76-79). Loss of apical root material is unpredictable and, when extending into the dentin, irreversible (80). Regarding the incidence of root resorption after orthodontic treatment there is no agreement in the literature, as the cited incidence varies from 22% to 100% . This variation probably relates to the use of different methods for identifying resorption. Killany reported that EARR of > 3mm occurs at a frequency of 30% in a patient population, while 5% of treated individuals have > 5mm of root resorption (81). Generally, clinical studies reveal a varied incidence, whereas histologic studies report a high incidence. The most frequent site is the apex, followed by mesial, buccal, distal and lingual surfaces. The maxillary incisors, particularly the central are the most prone to the process, followed by the maxillary molars and the canines. In the mandibular arch, the most prone teeth are the lateral and central incisors. Clinically, in most of the patients, root loss resulting from orthodontic treatment is minor and does not decrease the longevity or the functional capacity of the involved teeth (82, 83). However, a limited number of subjects may be more severely affected (84-86).

Brezniak and Wasserstein (87) classified root resorption according to severity as follows: a) cemental or surface resorption, where only the outer layers are
resorbed, to be fully regenerated or remodelled later b) dentinal resorption, with repair, where the cementum and the outer layers of dentin are resorbed, and are repaired along with morphological alterations c) circumferential root resorption, where full resorption of the hard tissue components of the root apex occurs, resulting in root shortening. Resorption defects are approximately 1mm in diameter and about 100µm in depth with mesial and buccal defects larger than distal and lingual defects. Most of the defects are limited to cementum, but about 30% penetrate into the dentine (88). Multinucleated giant cells with morphology identical to osteoclasts generate the resorption. Small defects approximately 15-20µm in diameter and up to 10µm deep may be caused by a single osteoclast, whereas larger craters result from groups of such cells.

The root resorption process
The process of root resorption is closely associated with injury and necrosis of the PDL. Histological studies have demonstrated the presence of small areas of surface resorption on the pressure side of all roots shortly after application of orthodontic forces as a consequence of the elimination process of the hyalinized tissue. The root resorbing cell, the odontoclast, shows similar cytologic and functional characteristics with the osteoclast (96, 97). Three stages are described in the hyalinized zone: a) degeneration ii) elimination of destroyed products and c) re-establishement. Human and animal research demonstrates that periodontal hyalinization precedes the root resorption process during orthodontic treatment (98-100). When an orthodontic force is applied over a long period of time, necrosis of the compressed PDL may develop. Macrophages are the first type of immune cells to leave the capillaries and enter the tissues, in sites of both PDL compression and tension. The leukocytes that migrate out of the capillaries include osteoclast progenitors that rapidly form multinucleated cells capable of dissolving mineralized tissues. In the case of EARR, the protective layer of cementoblasts interfacing the hyalinized PDL undergoes apoptosis, thus enabling odontoclasts to dissolve cementum and dentine. The protective layer of the cementoid is removed, thus the raw cemental surface is left attacked by the odontoclasts (98). According to Schwartz (101), when pressure decreases below a certain force, root resorption ceases and cementoid fills the resorbed lacunae. This process delays the
occurrence of new root resorption and initiates the healing process. Repair is described by migration of cementoblasts over the resorbed surface (102-104) and it is seen after 35 to 70 days after force application (105). After termination of active orthodontic treatment, some repair occurs, including smoothing and remodeling of cemental surfaces and the return of the PDL width to normal. However, the original root contour and length is never re-established (106).

Coronal section through the root of a premolar being moved to the left

Figure 5: PDL compression side to the left and tension to the right. A: dilatation of blood vessels and osteoblastic activity. B: osteoclasts removing bone are present. C: areas of beginning root resorption that will be repaired by later deposition of cementum. If resorption penetrates through the cementum and into the dentin, the result will be cementum repair that fills in craters in the dentin.


**Predisposing factors of root resorption**

The aetiology of orthodontically induced root resorption is complex: several factors, alone or in combination, may contribute to the initiation and progression of external root resorption. These include individual predisposing factors as involvement of genetic predisposition, systemic factors (ex. hormone deficiency), deviating root form, traumatized teeth with signs of root resorption before orthodontic treatment, adverse habits, age of the patient and stage of root formation at onset of treatment, the type of orthodontic appliances used, the type of tooth movement, the applied
forces, and treatment duration. Racial factors have been described, with Asians showing less resorption than whites and Hispanics (21, 107). Adults may experience greater amount of bone loss and root resorption than adolescents since their periodontal ligament is largely quiescent (108, 109). Initially, the magnitude of the orthodontic force and the rigid fixation of the archwire to the brackets were considered the most important factor predisposing a tooth to the root resorption. Jiggling forces and round tipping were also described as causes of orthodontic root resorption (110). Correlations were found between extraction treatment and root resorption (111, 112). Drugs, such as corticosteroids, alcohol, systemic diseases, such as asthma and allergies (107), previous history of dentofacial trauma and hypofunctional PDL, associated with nonoccluding teeth, have been proposed to predispose tooth roots resorption (113, 114). Recently, research is directed towards identifying genes associated to root resorption. Al Qawasmi et al (115, 116) reported an association between a linkage disequilibrium of IL-1β polymorphism in allele 1 and external root resorption. Recently, the system RANKL-RANK-OPG seems to play a major role in the genetic component associated with root resorption (117, 118).

**Effects of pharmacologic agents on root resorption**

In order to slow down orthodontically induced root resorption, some drugs, hormones and growth factors have been used (119-121). Igarashi et al (119) showed that bisphosphonates showed a dose-dependent reduction of root resorption when administered to rats. The hormone L-thyroxine increased the resistance of cementum and dentin to clastic activity, thus resulting in a reduction to the extent of root resorption (121). Recently, a positive effect of low-intensity pulsed ultrasound has been described on healing of orthodontically induced root resorption (122).

In conclusion, apical root resorption occurring in conjunction with conventional orthodontic treatment is an idiopathic and multifactorial problem, associated with both patient characteristics and treatment factors (90).
Diagnostic methods for root resorption

Radiographs are commonly used as a diagnostic tool for root resorption. The main radiographic methods are the orthopantomogram (OPG) and the periapical radiographs. However, orthodontically-induced root resorption after 7 weeks of treatment, verified histologically, is not visible in periapical radiographs (92, 123) and radiographic detection of apical root shortening requires a certain degree of resorption (124, 125). Thus, in film-based radiography, the diagnosis is uncertain during the first months of treatment. After 5-6 months a reliable radiographic diagnosis of apical root resorption can be performed.

In addition, the development of a standardized technique in order to compare the same teeth at different times is almost impossible especially when during the orthodontic movement the tooth is torqued or tipped. In order to obtain greater accuracy, digital subtraction radiography has been introduced (126-129). However, in spite of the great potential of digital reconstruction for more accurate analysis of root resorption, the method is time consuming and not easily clinically applicable (130).

The cone-beam computed tomography (CBCT) is a new radiographic method with application in several diagnostic areas, such as implant treatment, oral surgery, endodontic treatment, and temporomandibular joint imaging (131-134). The great advantage of this technology in that offers 3-dimensional (3D) imaging of dental structures and provides clear images of highly contrasted structures, such as bone (132). However, the diagnostic ability of CBCT in detecting orthodontically induced apical root resorption has not been sufficiently studied.

Thus, a diagnostic method available for identifying teeth at risk of severe resorption by a screening before the treatment, and for early detection of small root resorption during orthodontic treatment, is still inexistent.
2.3. Gingival crevicular fluid

2.3.1. Origin

In a healthy gingival crevice the initial fluid accumulated is a transudate of interstitial fluid, whereas under inflammatory conditions, becomes an exudate. This fluid is called gingival crevicular fluid (GCF) and is considered a serum-like exudate which bathes the gingival sulcus or periodontal pocket, and which follows an osmotic gradient with local tissues. The main route for GCF diffusion is through the basement membrane and then through the relatively wide intercellular spaces of the junctional epithelium. As this fluid traverses from the host microcirculation, through the tissues and into the gingival sulcus or pocket, it captures mediators involved in the destructive host response and byproducts of local tissue metabolism (135). The study of GCF was initially introduced in order to improve diagnosis, prevention and treatment of gingivitis and periodontitis. In the first cross-sectional and longitudinal studies, the amount of GCF was positively correlated to clinical parameters and gingival inflammation (136). Furthermore, studies were performed in order to investigate the composition of GCF and the relation between the concentration of its constituents and the periodontal health status (137). As a series of longitudinal clinical studies based on standardized periodontal indices showed that the progression of periodontal disease was chronic with episodes of active destruction and periods of remission (138), it was suggested that the analysis of GCF mediators could help to identify periodontal disease before any visible clinical inflammation. However, periodontal disease is multifactorial in nature and incorporates various etiological, host, environmental and genetic factors (139), thus making difficult the identification and validation of a single GCF constituent as prognostic marker of periodontal disease.

2.3.2. Collection

For the collection of GCF, several techniques have been described depending on the objectives of the study (135). The first method of collection was by glass capillary
tubes or micropipettes of known internal diameter and length. These are inserted into the entrance of the gingival crevice and the GCF will thus migrate into the tube by capillary action. The procedure may be repeated several times in the same crevice. The advantage of this method is that it allows the accurate volume determination of an undiluted “native” GCF sample. However, the technique may be traumatic and time-consuming as in order to collect an adequate volume of GCF the procedure should be repeated several times, the time may exceed 30 min. Furthermore, it is difficult to remove the complete sample from the tube.

By the Gingival washing method, a fixed volume of an isotonic solution is ejected in the gingival crevice and immediately re-aspirated. The process may be repeated 12 times to allow thorough mixing of the transport solution and GCF. The fluid collected represents a dilution of crevicular fluid. This technique is particularly valuable for harvesting cells from the gingival crevice, however not all the fluid can be recovered during the aspiration and re-aspiration procedure. Thus the method does not allow the accurate quantification of the GCF volume or composition, since the precise dilution factor cannot be determined (140).

By far, the most commonly used method for the collection of GCF is via filter paper strips. The method is quick, easy to use and atraumatic for the tissues. Furthermore, it can be applied to individual sites and allows the measurement of the volume collected. Two methods of collection have been described with the filter paper strips: the intracrevicular and the extracrevicular. In the former, the strip may be inserted either in the entrance of the crevice (141,142) or to the base of the pocket until “mild resistance” is felt. In the latter, the strips are overlaid on the vestibular surface of the tooth, marginal and attached gingivae (141). The volume of the fluid collected on the paper strip may be calculated either by weighing the tube before and after the collection procedure or by using a calibrated machine such as the “Periotron”, which measures and converts the electrical capacitance of the strips (143). The only disadvantage of the method is that the paper strips have a maximum capacity of 1µl which means that the duration of collection should be adapted in order not to exceed the maximum capacity of the strip (144).
2.3.3. Composition

The constituents of the gingival crevicular fluid derive mainly from the host and from the microorganisms in the supragingival and subgingival plaque. For many years, an important number of GCF components have been studied in correlation to the clinical measurements of periodontal disease, and/or in response to periodontal therapy (145-148). According to Armitage (149), more than 65 GCF components have been evaluated as potential diagnostic markers of periodontal disease progression. These markers may be divided in three main groups: host-derived enzymes and their inhibitors, inflammatory mediators and host-response modifiers and byproducts of tissue breakdown. Several useful reviews exist on the GCF host-derived markers and their potential diagnostic value in periodontitis (135, 144,150-157). Molecules, such as interleukin-1 beta (IL-1\(\beta\)), a potent bone-resorbing mediator able to stimulate osteoclast differentiation and activation (158) and IL-6, were found in elevated levels in gingiva and GCF from periodontitis sites (159-161). The cytokine tumor necrosis factor alpha (TNF-\(\alpha\)) secreted by monocytes and macrophages, able to induce the secretion of collagenase by fibroblasts and the resorption of cartilage and bone, has been also implicated in the destruction of periodontal tissue during periodontal disease (162). Prostaglandine E\(_2\), can not only mediate inflammatory responses such as increase in vascular permeability and dilatation, but also induces bone resorption through activation of osteoclastic cells. Alkaline phosphatase, beta-glucoronidase, cathepsin B, collagenese-2 (MMP-8) gelatinase, elastase as well as dipeptidyl peptidases II and IV have been studied as potential diagnostic markers for periodontitis (150). On the other hand, the reduction in inflammation resulting from effective periodontal treatment was associated with a reduction in the levels of most of these biochemical markers (146,163). Finally, GCF analysis has been performed in order to characterize the pharmacokinetic properties of locally-delivered systems used for the treatment of periodontal disease (164).

In conclusion, the study of GCF components has certainly contributed to our understanding of the role of the inflammatory response in periodontitis. However, the identification of one single diagnostic marker seems illusionary. The combination of several markers in the GCF together with the new improved technologies could contribute to improved periodontal diagnostics.
2.3.4. Composition changes during tooth movement

Research on the GCF composition and its role in periodontal pathology, has helped to broaden its role in relation to orthodontic tooth movement. After exposure to orthodontic forces, tissue fluid movement is followed by strain to cells and the extracellular matrix and local damage to the PDL. Cells in the PDL and alveolar bone are exposed to bioelectric signals, as well as to signal molecules derived from sensory nerve endings, from migratory leukocytes and from platelets. In addition, periodontal cells are stimulated to produce cytokines, growth factors and colony-stimulating factors that may function as autocrines or paracrines. The outcome of this physical and chemical perturbation is tissue remodeling and tooth movement. This process is further reflected in the synthesis and secretion of various components resulting in modification of GCF composition (165). To monitor the biological responses to orthodontic forces in humans, analyses of various cell mediators or enzymes found in the GCF, have been proposed. The induction of these markers comes from the compression of the periodontal ligament after application of the orthodontic force, in levels sufficient enough to be diffused into the GCF. Until now, an important number of GCF constituents has been studied in relation to the events taking place during orthodontic tooth movement (166, 167). For example, osteocalcin (168), transforming growth factor beta (169), acid and alkaline phosphatase (170,171), cathepsin B (172), necrosis factor alpha (173,174), epidermal growth factor, beta-2-microglobulin (169), glycosaminoglycans components (175, 176), prostaglandine E\textsubscript{2} (68) as well as lactate dehydrogenase activity (177) and aspartate aminotransferase activity (178) have been found to be significantly elevated in teeth undergoing orthodontic movement, as compared to untreated controls. The group of interleukins in the GCF has attracted special attention: the levels of interleukin-1 beta (179-182), interleukin-8 (181,183), interleukins 2 and 6 (181) have been shown to increase during orthodontic treatment. Finally, several matrix metalloproteinases and their inhibitors have been studied in relation to orthodontic tooth movement (184-186). Most of the studies have generally shown that peak levels of substances occur on average 1 to 2 days after the application of the stimulus and return to baseline values after about 1 week.
The receptor activator of nuclear factor-kappa ligand (RANKL) has been identified recently as a member of the membrane-associated tumor necrosis factor ligand family and is an important regulatory molecule of osteoclastogenesis (187, 188). Vernal et al (189) showed that the total amount of RANKL in GCF is significantly increased in association with periodontal disease and Mogi et al (190) found that the ratio of the concentration of RANKL to that of OPG in the GCF was significantly higher in periodontal disease patients than in healthy subjects. It has been suggested that the RANKL/OPG system plays an important role in the development of periodontal disease. Recently, the role of RANKL and its inhibitor OPG in inducing alveolar bone remodeling during orthodontic tooth movement, has been demonstrated (191-197). Interestingly, Kawasaki et al (193) by comparing the levels of RANKL and OPG in the GCF of teeth during orthodontic tooth movement, found that the average amount of tooth movement for juveniles was larger than that for adults. This difference was associated with a decrease in the RANKL/OPG ratio in GCF. The levels of the rate of orthodontic tooth movement have been shown to increase significantly by the transfer of RANKL gene (198) and on the contrary to decrease significantly by the transfer of OPG gene (199, 200).

In brief, all these studies have suggested that the GCF mediators levels analysis reflect the biological activity that takes place in the periodontium during orthodontic movement. However, we have to point out that significant heterogeneity was found between many of these studies, probably related to different GCF sampling and analysis procedures. Therefore, it is suggested that future studies should focus on, amongst others, refinement of the GCF sampling and measuring protocols and the relationship between mediators production and force re-activation, in order to provide a better illustration of the high potential of GCF as a diagnostic tool to monitor clinical outcome in orthodontics (166).
2.4. Unresolved questions/Aim of this Research

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by the remodeling of the periodontal ligament and alveolar bone. The remodeling activities and the ultimately tooth displacement are the consequence of an inflammatory process. Vascular and cellular changes were the first events to be recognized and described. With the advancement of research techniques an important number of inflammatory mediators, growth factors, neuropeptides and metabolites of arachidonic acid have been detected in the surrounding periodontal tissue. Most of them are produced in sufficient amounts to diffuse into the gingival crevicular fluid and their analysis has been used to evaluate the local cellular metabolism that accompanies bone remodeling process during orthodontic tooth movement. Gingival inflammation, discomfort or pain and root resorption are common undesirable side effects of orthodontic treatment. Gingival health is compromised during orthodontic treatment because of alterations in the composition of bacterial plaque and consequently the development of gingivitis, especially when fixed orthodontic appliances are used. Discomfort or pain is reported by most patients during the first day or couple of days of treatment and comes from the compression of periodontal ligament after application of the orthodontic force. As for root resorption, it has been reported that almost all roots, shortly after the application of orthodontic force present histologically small areas of surface resorption. In most cases this resorption does not decrease the functional capacity of the involved teeth, but in other cases the degree of resorption may become important, resulting in an unfavourable crown/root ratio and thus less periodontal support for the tooth, decreasing its longevity.

The above points lead to several questions:

- What are the clinical and biochemical characteristics accompanying the inflammatory process of periodontal tissues around teeth subjected to forces by fixed orthodontic appliances?
- Does the composition of gingival crevicular fluid change in relation to the type of force exerted on the periodontium (tension or compression) during the early phase of orthodontic treatment?
• Does a common orthodontic procedure such as the placement of elastic separators result in discomfort or pain to the patients and is there an association between the expression of pain-related molecules in the GCF and the perceived intensity of pain?

• Do the standardized clinical periodontal parameters obtained during orthodontic treatment could be related to the cervical root resorption observed after treatment?

• What is the diagnostic accuracy of digitized periapical radiographs in detecting orthodontically induced apical root resorption?

• Does the use of orthopantomograph can help the clinician to make the decision on continuation and/or possible modification of orthodontic treatment because of orthodontically induced root resorption?

The studies described in this Thesis were designed to address these questions.

We previously reported that gingival health is compromised during orthodontic treatment and that inflammation in periodontal tissue is almost inevitable, especially when fixed orthodontic appliances are used. Oral hygiene becomes difficult even for the most motivated patients. The effect of fixed orthodontic appliances on marginal periodontal tissue has been mainly studied by means of clinical and microbiological studies. In Chapter 3, we show that not only clinical but also biochemical changes accompany the inflammatory process of periodontal tissues around fixed orthodontic appliances. Children and adolescents under orthodontic treatment, presented gingival overgrowth, bleeding sites and significantly higher levels of GCF inflammatory mediators as compared to a group of matched-aged subjects without fixed orthodontic appliances.

During orthodontic treatment, the tooth moves in the periodontal space by generating a “pressure side” and a “tension side”. In the “pressure or compression side”, the PDL displays disorganization and diminution of fiber production, leading to a vascular constriction and a decrease of cell replication. On the “tension side”, the PDL fiber bundles are stretched and this stimulation leads to enhanced proliferative activity. In Chapter 4 we evaluated whether the different stresses exerted on the periodontium by the tooth movement (tension or compression) are also reflected by
differences in the GCF composition changes. We found that a simple routine procedure, such as the placement of elastic separators, can change the composition of GCF, at least at the level of IL-1β, SP and PGE₂ expression, and that these changes depended on whether tension or compression forces were exerted.

Discomfort or pain is a very common undesirable side effect of orthodontic treatment. Pain during orthodontic treatment, occurs mainly during the first day or first couple of days of treatment and its intensity falls to normal levels after 7 days. The experience of pain varies substantially among subjects, depending on gender, personality and especially previous general and dental experience. In Chapter 5, firstly we evaluated the experience of pain perceived by the patients during a common orthodontic treatment procedure, such as the placement of elastic separators and secondly we studied the effect of this procedure on IL-1β, SP and PGE₂ levels in GCF. Pain intensity as evaluated by the VAS scale increased after 1h, remained high on day 1, and had decreased by day 7. Changes in the composition of GCF occurred immediately after the placement of separators for IL-1β and for SP and PGE₂, after 1 day. Associations were found between the intensity of pain and the GCF mediators levels.

In Chapters 6 and 7, we aimed to investigate the presence and severity of root resorption in teeth that had undergone experimental orthodontic treatment. Root resorption is a common, undesirable side effect of orthodontic tooth movement. When a tooth is tipped buccally, pressure is created on the cervical part of the buccal side of the tooth and on the palatal/lingual side. These pressure areas are the ones where root resorption is mostly expected. We performed an experimental orthodontic treatment in 16 patients who presented severe crowding in both jaws and needed premolar extractions. Half of the premolars underwent a standardized orthodontic movement for 8 weeks, the other half served as controls. At the end of this period, all premolars were carefully extracted and scanned in a micro-computed tomography (CT) scanner for evaluation of presence and severity of root resorption. In Chapter 6 we aimed to assess whether there is a relationship between clinical periodontal parameters obtained during orthodontically induced tooth movement and the resorption located on the cervical part of the root, observed after treatment. Standardized clinical parameters, such as Plaque Index (PI), Gingival Index (GI), periodontal pocket depth (PPD) and presence or absence of bleeding on probing
(BOP), were obtained several times during the experimental tooth movement. We found that almost all of the experimental teeth and only one of the control teeth showed signs of cervical root resorption. However, no changes were observed clinically between the experimental and control teeth during the orthodontic movement, thus leading to the conclusion that periodontal parameters are not associated with root resorption.

The diagnostic accuracy of digitized periapical radiographs to detect apical root resorption during orthodontic tooth movement was explored in Chapter 7. Radiography is the only method used in the every-day practice for diagnosing root resorption. Several radiographic methods have been used, but to date the recommended tool is periapical radiography. However, a certain degree of resorption in the apex of the root is required before being detectable on the radiograph. Using the micro-CT scanner as criterion standard (gold standard), we provided evidence that apical root resorption is underestimated when evaluated by digitized periapical radiography. In fact, the Rx method showed a low sensitivity of 44%, which means that less than half of the teeth presenting root resorption as identified by the micro-CT scanner, were also identified by radiography.

Based on the previous results on the limitation of periapical radiography to detect apical root resorption, in Chapter 8 we investigated the diagnostic ability of the OPG in detecting orthodontically induced apical root resorption, by using the cone-beam computed tomography (CBCT) as a criterion standard. The CBCT is a relatively new radiographic method, having the great advantage of offering a 3-dimensional (3D) imaging of dental structures. In the field of Dentistry, the method has been used mainly for implant treatment and oral surgery. In the present study we evaluated the presence and severity of apical root resorption by panoramic radiography (OPT) and CBCT in 275 teeth in 22 patients near the end of orthodontic treatment with fixed appliances. We found that OPT underestimated apical root resorption, as significant differences were observed between the two methods. We concluded that OPT is not a powerful tool for detecting apical root resorption as compared to CBCT, but at the same time has a less medical risk. Therefore, for the moment the use of CBCT should be limited for precise reasons.
3. Detection of gingival crevicular fluid cytokines in children and adolescents with and without fixed orthodontic appliances

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Detection of gingival crevicular fluid cytokines in children and adolescents with and without fixed orthodontic appliances

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Abstract
Objective. To study the expression of IL-1β, IL-4, and IL-8 in the gingival crevicular fluid (GCF) of children, adolescents, and young adults with and without fixed orthodontic appliances. Material and methods. Eighty systemically healthy children and adolescents participated in the study: 56 aged between 8 and 16 years without any orthodontic appliance (Group A) and 24 aged between 10 and 20 years having worn fixed orthodontic appliances for at least 12 months (Group B). Clinical examination included presence or absence of plaque, probing depth, bleeding on probing, and gingival overgrowth. GCF was collected by means of Durapore strips from four randomly selected sites per subject. The contents of interleukin-1 beta (IL-1β), interleukin-4 (IL-4), and interleukin-8 (IL-8) were detected by ELISA, measured as total amounts (pg/30s) and expressed in log scale. Results. Statistically significant differences were noted for the mean log IL-1β, IL-4, and IL-8 between the two groups: Group B showed significantly higher mean levels in log IL-1β and log IL-8 compared to Group A. Mean levels of log IL-4 were lower in Group B, although they did not reach statistical significance. Furthermore, mean levels of log IL-1β and log IL-8 were associated with bleeding sites (p < 0.001) and gingival overgrowth, while mean level of log IL-4 was associated with non-bleeding sites and no gingival overgrowth (p = 0.001). Conclusion. Our findings suggest that fixed orthodontic appliances result in an increase in the expression of IL-1β and IL-8. This may reflect biologic activity in the periodontium during orthodontic tooth movement.

Key Words: Cytokines, gingival crevicular fluid, orthodontic treatment

Introduction
Gingival health is compromised during orthodontic treatment. A local change is produced in the oral ecosystem altering the composition of bacterial plaque and resulting in the development of an inflammatory process [1–3]. Inflammation in periodontal tissue is almost inevitable when fixed orthodontic appliances are used [4,5]. These may render oral hygiene difficult even for the most motivated patients [6]. In the past, the effects of fixed orthodontic appliances on marginal periodontal tissues were analyzed predominantly by means of clinical and microbiological studies [7]. Clinically, an increase in plaque scores, gingival scores, and probing depths has been reported [1,8]. These clinical observations are attributed to the alteration of the composition of the bacterial biofilm occurring soon after the placement of fixed orthodontic appliances. In most cases, there is a shift in the subgingival microflora to a periopathogenic population with increases in anaerobic rods and reductions in facultative anaerobes [9–11].

Another way of studying the changes occurring during orthodontic tooth movement is by analysis of the composition of the gingival crevicular fluid (GCF). GCF reflects the immune and inflammatory reactions deriving from host-parasite interactions [12,13] and bio-mechanical stress [14–17]. It is a non-invasive method, and until now a wide variety of substances involved in bone remodeling, and produced by the PDL cells in sufficient amounts to diffuse into the GCF, has already been studied [18]. Among many inflammatory and immune mediators identified in GCF, cytokines have attracted particular attention. For example, the level of interleukin-1 beta (IL-1β), a known potent cytokine produced...
mainly by activated monocytes increases significantly during inflammation [19] and participates in the initiation of bone resorption [20,21]. Interleukin-4 (IL-4), originally described as B-cell growth factor, is a potent downregulator of macrophage function. Localized absence of IL-4 in diseased periodontal tissues was associated with periodontal disease activity and progression [22]. Finally, Interleukin-8 (IL-8) is produced by a wide variety of cells (polymorphonuclear leukocytes, monocytes, macrophages, and fibroblasts) and plays a key role in the accumulation of leukocytes at the sites of inflammation [23].

The aim of the present study was to evaluate the clinical and biochemical characteristics accompanying the inflammatory process of periodontal tissues around fixed orthodontic appliances in children, adolescents, and young adults. The levels of IL-1β, IL-4, and IL-8 in GCF of children undergoing orthodontic treatment are compared to those without orthodontic appliances.

Material and methods

Sample size

An allocation ratio of 1:2 was used for better representation of the wide range of age groups. A sample size of 23 and 46 subjects for each group, respectively, achieves 90% power to detect a pattern of IL-1β and IL-4 means, equal to 2.2 and 2.5 log × pg/30s with a standard deviation of 0.35 log × pg/30s and level of significance equal to a = 0.05. Similarly, for IL-8, a pattern of means equal to 3.2 and 3.5 log × pg/30s will be detected with the same number of subjects for each group, respectively, and under the same assumptions.

Subjects

A total of 80 healthy children, adolescents, and young adults participated in the study. They were selected from two private dental clinics in Piraeus, Greece limited to pedodontics and periodontics, respectively. All were systemically healthy and had not received antibiotics in the 6 months prior to entering the study. Before the beginning of the study, all subjects underwent a session of supragingival scaling and received oral hygiene instructions. On the day of the examination they presented no non-treated carious lesions. The subjects were divided into two groups: Group A of 56 children and adolescents (32 M, 24 F, mean age 12.3±2.9) with healthy periodontium showing no radiographic evidence of bone loss and a probing pocket depth <2 mm. Group B of 24 adolescents and young adults (15 M, 9 F, mean age 15.1±2.9) wearing fixed orthodontic appliances for at least 12 months and showing no radiographic evidence of bone loss.

The qualified subjects were entered consecutively in the study. All participants (parents of children and adolescents) gave their consent to participation. The study was conducted in accordance with the principles outlined in the Helsinki declaration of 1975 (as revised in 1983) on experimentation involving human subjects.

Periodontal examination

The clinical evaluation was performed by one periodontist (J.K.) and included the presence or absence of plaque (PL) [24], assessment of probing pocket depth (PPD), and bleeding upon probing (BOP) [25] at four sites around each tooth, excluding 3rd molars. The presence or absence of inflammatory gingival overgrowth (GO) was also assessed. Measurements of PPD were carried out to the nearest millimeter using a Goldman/Fox Williams periodontal probe. The number of teeth present was recorded for each child.

Investigator calibration

A total of 10 periodontally healthy adolescents and young adults who were not included in the study group were used for the calibration evaluation. The single designated examiner (J.K.) performed full mouth PPD and CAL measurements for all 10 subjects and repeated the measurements after 15 min. The intra-examiner STD for repeated measures was 0.1 mm. Examiner’s reproducibility was 99.8%.

Gingival crevicular fluid sampling

All teeth within a quadrant were numbered from 1 to 7. One tooth and the respective mesial or distal site from each quadrant were randomly sampled using the random number generator of SPSS 13.0. The GCF was collected from four sites in each child–adolescent–young adult by means of Durapore filter membranes (pore size = 0.22 mm; Millipore Corp., Bedford, Mass., USA). After isolation of the test sites from saliva, a first Durapure strip was inserted 1 mm into the sulcus and left in place for 15 s. Three minutes after removal of the first strip, a second Durapure strip was similarly inserted in the same site for 15 s. The two strips were then placed in a microcentrifuge tube and immediately frozen at −20°C until the day of the analysis. In the event of visible contamination with blood, the strips were discarded.

Clinical measurements were recorded and GCF sampling sites were pre-selected one week before sampling. Clinical parameters were registered again after GCF sampling and these values were used in the analysis.
Analysis of cytokine production

The samples were transferred to and analyzed blindly by C.G. in the Department of Periodontology at the University of Geneva. The contents of IL-1β, IL-4, and IL-8 were measured at each of the four sites from each patient. A total of 320 samples in 80 subjects were analyzed. On the day of the analysis, 350 µl of phosphate-buffered saline (PBS, pH 7.2) was added to the tubes containing the strips. The strips were gently shaken for 1 min and then centrifuged at 2000g for 5 min, with the strips kept at the collar of the tube in order to elute GCF components completely. After strip removal, the supernatant was divided into three aliquots for determination of each biochemical compound. The amounts of IL-1β, IL-4, and IL-8 were determined by enzyme-linked immunosorbent assays (ELISA) specific for each compound (Ruwag Diagnostics, Zürich, Switzerland). The assays were carried out in accordance with the manufacturer’s instructions and the levels of the biochemical compounds were reported as total amount (pg) per 30 s sample and were expressed in log scale.

Total cytokine amounts per 30 s samples were calculated based on ELISA concentration values. Sites with cytokine levels below the limits of the assay’s detectability were scored as 0 pg.

Data analysis

Statistical analyses were carried out on the full set of patients who participated in the study. Baseline demographic and clinical characteristics were summarized by the mean and standard deviation (±SD) for continuous measures and the number and percent for categorical variables. Differences of demographic data and probing depth between groups were tested using a t-test. Differences between genders and differences in plaque, bleeding on probing, and gingival overgrowth levels between groups were tested using the chi-squared test. A mixed model analysis for the logarithms of IL-1β, IL-4, and IL-8 was made using a random intercept for describing the correlation between observations from the same subject. Differences in log IL-1β, log IL-4, and log IL-8 means between groups, genders, bleeding on probing, and gingival overgrowth were tested using appropriate F-tests based on REML. However, the F-test degrees of freedom are not presented since they are taken into account from the corresponding p-values.

Results

Demographic and periodontal variables

The demographic and periodontal variables in each group are summarized in Table I. As expected, the mean PPD, the percentage of sites with bleeding on probing, and the presence of gingival overgrowth were significantly higher in subjects wearing orthodontic appliances (Group B) than in those without (Group A). The percentage of sites with plaque accumulation did not differ between the two groups.

Means of log IL-1β, IL-4, and IL-8 in Groups A and B

The mean (SD) total amounts of IL-1β, IL-4, and IL-8 in the two groups are given in Table II and the fitted log transformed means (SE) in Table III. Statistically significant differences were found for the mean log of IL-1β and IL-8 between the two groups (F = 33.262, p < 0.001 and F = 4.054, p = 0.048, respectively). The mean log of IL-4 was not statistically different between the groups (F = 3.074, p = 0.083).

No differences were noted between males and females for log IL-1β (F = 1.979, p = 0.163), for log IL-4 (F = 3.158, p = 0.079), or for log IL-8 (F = 3.194, p = 0.078) (data not shown in the tables).

Correlations with bleeding sites

Table IV gives possible associations between the mean log of IL-1β, log IL-4, and log IL-8 scores and BOP levels. Log IL-1β and log IL-8 were associated with the bleeding sites (p < 0.001), whereas IL-4 was associated with non-bleeding sites (p < 0.001).

Correlations with gingival overgrowth

Table V gives possible associations between the mean log IL-1β, log IL-4, and log IL-8 scores and presence or absence of gingival overgrowth expressed as 1 and 0, respectively. IL-1β and IL-8 were associated with gingival overgrowth (p = 0.018 and p < 0.001, respectively), whereas IL-4 was not.

Table I. Demographic and periodontal variables.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>8.16</td>
<td>10.20</td>
</tr>
<tr>
<td>Mean (SD) age, years</td>
<td>12.3 (2.9)</td>
<td>15.1 (2.9)</td>
</tr>
<tr>
<td>Men/women</td>
<td>32/24</td>
<td>13/11</td>
</tr>
<tr>
<td>Mean probing depth (SD) at sampling sites</td>
<td>0.03 (0.19)</td>
<td>0.38 (0.40)**</td>
</tr>
<tr>
<td>Plaque1</td>
<td>71 (5.8)</td>
<td>68.8</td>
</tr>
<tr>
<td>Bleeding on probing1 (BOP)</td>
<td>17 (6.5)</td>
<td>64.6#</td>
</tr>
<tr>
<td>Gingival overgrowth1 (GO)</td>
<td>9.4 (5.6)</td>
<td>76#</td>
</tr>
</tbody>
</table>

1Percent positive sites. 2Chi-squared, among groups < 0.001.
associated with sites without gingival overgrowth ($p < 0.001$).

**Discussion**

In the present study, the levels of IL-1β, IL-4, and IL-8 were monitored in the GCF of orthodontically treated young subjects and untreated controls. Total amounts rather than concentrations were used, because very small errors in volume determination can lead to large errors in estimates of final concentrations if the total volume collected is small. The results demonstrate that young adults with fixed orthodontic appliances had significantly higher levels of IL-1β and IL-8 compared to children and adolescents with healthy periodontium. The levels of IL-4 did not differ between the two groups. These differences between the groups were associated with the presence of bleeding on probing and the presence of inflammatory gingival overgrowth.

Oral hygiene was monitored before the day of examination. Ideal oral hygiene was important because the study aimed to investigate the differences on the levels of IL-1β, IL-4, and IL-8 in GCF, as a consequence of inflammation caused by tooth movement rather than plaque accumulation. In fact, plaque accumulation did not differ between the groups.

Several studies have reported that, during orthodontic treatment with a fixed appliance, an increase in the plaque accumulation and gingival inflammation is seen frequently. This can be attributed to the increased difficulty of effective cleaning around the appliance [5,6]. Regardless of the level of oral hygiene, when a fixed appliance is placed the majority of patients develop generalized gingivitis [26,27]. Histologically, the interdental gingival of banded teeth presents the pattern of an established gingival lesion: pronounced leukocyte infiltration and inflammatory exudation in the area of the transepithelial fibers [28].

**Table IV. Differences (δ) between BOP levels in the mean log IL-1β, log IL-4, log IL-8.**

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>IL-4</th>
<th>IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ BOP (1-0)</td>
<td>0.272</td>
<td>−0.227</td>
<td>0.260</td>
</tr>
<tr>
<td>F</td>
<td>35.092</td>
<td>20.937</td>
<td>64.983</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BOP = bleeding on probing. 0 = BOP negative. 1 = BOP positive.

Furthermore, gingival enlargement occurs soon after placement of a fixed orthodontic appliance and explains the increase of probing depth found during treatment [8]. In our study, a significant increase in PPD was seen in the orthodontically treated patients. This increase could more properly be related to moderate gingival enlargement rather than attachment loss. It is well established that orthodontic appliances can influence the subgingival microbial population, i.e. “shifting” it to a more pathogenic population. This may in part explain the inflammation during orthodontic treatment even in patients with excellent plaque control [29].

In terms of the GCF metabolites, several studies have shown that during orthodontic treatment the application of mechanical force induces an inflammatory reaction by the compression of periodontal ligament [30–32]. As a result, both the GCF flow and its components are modified. Until now, a wide variety of substances have already been studied [18]. In our study, the differences on the GCF cytokine levels observed between the two groups reflect the events taking place in the periodontium during orthodontic tooth movement. Furthermore, these differences may reflect the age-associated changes in immunity, since there is a communication between the endocrine and immune system that may determine the expression of cytokines in puberty [33]. However, only a few studies have considered age status as a modifying factor for the variations in intracellular cytokine production. This variation was observed when comparing younger and aging adults or children and adolescents [33,34].

It has been proposed that the severity of gingivitis in childhood is linked to several factors, including puberty [35]. However, so far there is no evidence of a direct link between gingivitis and puberty. This is because the chronological age is a poor indicator of puberty, the measurement of gingivitis is subjective, and the hormone levels are not measured.

In conclusion, within the limitations of the study, the results indicate that fixed orthodontic appliances, as well as puberty, result in an increase in the expression of IL-1β and IL-8 which might reflect the host response to the orthodontically induced local inflammation.
Acknowledgment

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


[34] Kowalczyk D, Baran J, Webster AD, Zembala M. Intracellular cytokine production by Th1/Th2 lymphocytes and monocytes of children with symptomatic transient hypogammaglobulinemia of infancy (THI) and selective IgA deficiency (SigAD). Clin Exp Immunol 2002;127:507-12.

4. Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides

A. Dudic, S. Kiliaridis, A. Mombelli, C. Giannopoulou
Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides


The aim of this study was to evaluate whether the application of tension or compression forces exerted on the periodontium during the early phase of orthodontic tooth movement is reflected by differences in the composition of the gingival crevicular fluid (GCF), at the level of interleukin-1β (IL-1β), substance P (SP), and prostaglandin E₂ (PGE₂). Eighteen children (mean age 10.8 yr) starting orthodontic treatment were included in the study. Molar elastic separators were inserted mesially to two first upper or lower molars. One of the antagonist molars served as the control. GCF was collected from the mesial and distal sites of each molar, before (−7 d, 0 d) and immediately after (1 min, 1 h, 1 d, and 7 d) the placement of separators. The levels of IL-1β, SP, and PGE₂ were determined by enzyme-linked immunoassay assay. At the orthodontically moved teeth, the GCF levels of IL-1β, SP, and PGE₂ were significantly higher than at the control teeth in both tension and compression sides, and at almost all occasions after insertion of separators. The increase, relative to baseline values, was generally higher in tension sides. For the control teeth, the three mediators remained at baseline levels throughout the experiment. The results suggest that IL-1β, SP, and PGE₂ levels in the GCF reflect the biologic activity in the periodontium during orthodontic tooth movement.

During orthodontic treatment, periodontal tissues respond rapidly to mechanical stress with consequent metabolic changes that allow tooth movement. One way of evaluating these changes is by analysis of the gingival crevicular fluid (GCF) composition. This non-invasive and simple method has been used to investigate the cellular response of the periodontal ligament (PDL) during orthodontic treatment (1). A variety of substances involved in bone remodelling and produced by the PDL cells in sufficient quantities to diffuse into the GCF, have already been studied (2). Changes in the composition of GCF as consequence of bacterially induced inflammation has also been evaluated (3). The mechanism of bone remodelling during orthodontic treatment is related on the one hand to the release of inflammatory mediators, such as prostaglandin E₂ (PGE₂) and interleukin-1β (IL-1β), and on the other hand to the production of neuropeptides, such as substance P (SP). In a previous publication we reported that initial orthodontic tooth displacement by the use of separators induces pain and a rapid release of these three GCF biochemical mediators. Furthermore, associations were found between pain intensity and the levels of PGE₂ and IL-1β, 1 h and 1 d after the placement of separators, respectively (4).

The level of IL-1β, a known potent cytokine produced mainly by activated monocytes, increases significantly during inflammation (5) and it participates in the initiation of bone resorption (6, 7). SP is a multifunctional neuropeptide able to stimulate bone resorption activity of osteoclasts and to modulate emotional stress. The levels of SP in GCF have been found to be significantly higher in sites showing periodontal inflammation (8), and decreased levels of SP were associated with the reduction of inflammation after efficient periodontal treatment (9). PGE₂ is able to mediate inflammatory responses and induce bone resorption through the activation of osteoclastic cells. Higher levels of PGE₂ were found in the GCF of patients with periodontitis than in healthy controls (10) and in the GCF of teeth undergoing orthodontic movement (11). For example, UEMATSU et al. (12) studied the levels of several inflammatory...
mediators, such as IL-1β, interleukin-6, tumor necrosis factor (TNF), epidermal growth factor and β2-microglobulin, in the GCF of teeth undergoing orthodontic tooth movement. Significant elevations were observed in the experimental teeth as compared to control teeth for all biochemical parameters. Similar results were reported by Griev et al. (11) for PGE2 and IL-1β. However, these studies do not relate the levels of GCF biochemical markers to the type of stress exerted on the periodontium (tension or compression).

Therefore, in the present study the levels of IL-1β, SP, and PGE2 were analyzed in the GCF of the mesial and distal sites of teeth during the early phase of orthodontic movement. Comparisons between the distal and mesial sites were assessed to evaluate whether the different stresses exerted on the periodontium by the tooth movement (tension or compression) are reflected by differences in the GCF composition changes.

Material and methods

Eighteen young subjects (8 female and 10 male; age range 8.9-13.8 yr) participated in this study. Patients attended the department of orthodontics of our University for comprehensive orthodontic treatment because of crowding in one or both jaws. Second molars were not yet erupted. Further inclusion criteria were: (i) good general health; (ii) lack of antibiotic therapy within the last 6 months; (iii) no use of anti-inflammatory drugs in the month preceding the study; and (iv) healthy periodontium with generalized probing depth ≤ 3 mm and no radiographic evidence of bone loss. Informed consent in written form was obtained from the patients and the parents before starting the study. The protocol was reviewed and approved by the local Medical Ethics Committee of our University.

The outline of the study is shown in Fig. 1: 1-2 wk before placement of orthodontic separators (~14 d), all subjects underwent a session of supragingival scaling and received oral hygiene instructions to reach a level of meticulous plaque control. Remotivation was performed during the whole study, if necessary. During the second visit (~7 d) and at 0, 1, and 7 d afterwards, the following periodontal parameters were assessed at four sites in three first molars: plaque index (PI) (13); gingival index (GI) (14); periodontal probing depth (PPD); and the absence of bleeding within 15 s after probing (BOP). During the third visit (0 d), orthodontic elastic separators were inserted mesially to the first upper or lower molars, the antagonist left or right first molar serving as control. GCF sampling was performed before (~7 d, 0 d) and after (1 min, 1 h, d, 7 d) the placement of separators.

GCF sampling

GCF was collected by means of Durapore filter membranes (pore size: 0.22 μm; Millipore, Bedford, MA, USA) from the mesial and distal sites of the two experimental molars, where the elastic separators were inserted, and from the mesial and distal sites of the control molars. The collection was assessed from the buccal side of each molar using a previously established standardized method (15). Briefly, each tooth included in the study was isolated with cotton rolls, any supragingival plaque was removed with cotton pellets, and the tooth surface was dried gently with air. The strip was then inserted 1 mm into the sulcus and left in situ for 20 s. After collection, it was placed in a microcentrifuge tube and immediately frozen at ~70°C until the day of the analysis.

Analysis of GCF mediators production

The contents of IL-1β, SP, and PGE2 were measured at each of the six sites where GCF was collected from each patient. A total of 648 samples were analyzed. On the day of the analysis, 350 μl of phosphate-buffered saline (PBS, pH 7.2) was added to the tubes containing the strips. The strips were gently shaken for 1 min and then centrifuged at 2000 g for 3 min, with the strips kept at the collar of the tube in order to elute GCF components completely. After strip removal, the supernatant was divided into three aliquots for the determination of each biochemical compound. The amount of IL-1β, SP and PGE2 was determined by enzyme-linked immunosorbent assays (ELISA) (16) specific for each compound (Ruwag Diagnostics, Zurich, Switzerland; and Alexis, Lausen, Switzerland). The assays were carried out in accordance with the manufacturer’s instructions and the levels of the biochemical compounds were reported as total amount (pg) per 20 s sample.

Statistical analysis

For each individual, the experimental mesial sites, the experimental distal sites and the respective control sites were used to create the experimental and control groups: two experimental and two control groups of sites were created, as follows: experimental-mesial sites (E-M); experimental-distal sites (E-D); control-mesial sites (C-M); and control-distal sites (C-D). The paired t-test was used to test the hypothesis that no difference existed between the experimental and control groups in each of the six sampling occasions. Longitudinal changes for each biochemical parameter at each site (mesial and distal) were expressed as a proportion of the baseline value obtained from the mean of ~7 d and 0 d in the same sites. The paired t-test was also used to compare the relative changes between the measures.
Methodologic error and intro-individual variations: clinical and biochemical parameters with the number of observations, mean of values (−7 d and 0 d) and of the differences (−7 d minus 0 d); the standard deviation (SD) of the mean differences, the P-values of the paired t-test, the coefficient of reliability (for the biochemical data), and the coefficient of variation (for the clinical data)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean values of −7 d</th>
<th>Mean values of 0 d</th>
<th>Means of differences (−7 d, 0 d)</th>
<th>SD of the means of differences</th>
<th>P-value</th>
<th>Coefficient of reliability</th>
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<td>0.33</td>
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<td></td>
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<tr>
<td>IL-1β</td>
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<td>4.56</td>
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<td>0.31</td>
<td>0.75</td>
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<tr>
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<td>2.27</td>
<td>2.36</td>
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<td>0.66</td>
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<tr>
<td>PGE2</td>
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<td>4.42</td>
<td>4.37</td>
<td>0.01</td>
<td>0.33</td>
<td>0.33</td>
<td>0.94</td>
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</tbody>
</table>
| **BOP**: bleeding on probing; GI: gingival index; IL-1β, interleukin-1β; PGE2, prostaglandin E2; PI1, plaque index; PPD, pocket probing depth; SP, substance P.**

<table>
<thead>
<tr>
<th></th>
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<th>PGE2</th>
</tr>
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<tr>
<td>E-M</td>
<td>C-M</td>
<td>P-value</td>
<td>E-M</td>
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<tr>
<td>−7 d</td>
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<td>3.8 ± 2.3</td>
<td>0.823</td>
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<tr>
<td>0 d</td>
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<td>3.8 ± 2.1</td>
<td>0.819</td>
</tr>
<tr>
<td>1 min</td>
<td>5.7 ± 2.7</td>
<td>3.8 ± 2.3</td>
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<tr>
<td>1 h</td>
<td>8.0 ± 4.1</td>
<td>3.8 ± 2.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1 d</td>
<td>8.0 ± 4.1</td>
<td>3.7 ± 2.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>7 d</td>
<td>5.8 ± 2.4</td>
<td>3.6 ± 2.0</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Results are expressed as pg per 20 s sample.

of the experimental teeth at 1 min, 1 h, 1 d, and 7 d. Furthermore, the Wilcoxon paired signed rank test was employed to detect significant differences between mesial and distal sites, at each time point.

The statistical analysis was determined using spss for Windows (Release 13.0.0, standard version; SPSS, Chicago, IL, USA).

Methodologic error and intradividual variation

The measurements of one site in 18 subjects (mesial site of experimental tooth) from 7 d and 0 d were used to evaluate the systematic error and the intradividual variation (Table 1). The coefficient of variation (in the same site for clinical parameters) and the coefficient of reproducibility (for biochemical parameters) were calculated to test the methodologic error, not excluding the interference of the intra-individual variation. The findings indicated that the reproducibility between these time points was sufficient to evaluate changes occurring during the experimental trial.

Results

At baseline, no significant differences on any of the clinical parameters were observed between experimental and control sites. Both control and experimental sites showed good periodontal status, with PI1 and GI scores in the range of 0 to 1 and a pocket depth of 3 mm or less. The differences between the site groups (E-M vs. C-M, E-D vs. C-D, and E-M vs. E-D) for each variable were not statistically significant (data not shown). All 18 participants maintained good oral hygiene throughout the study. No significant change in PI1, GI, PPD or BOP was found at any time or at any site.

GCF IL-1β, SP, and PGE2 levels in mesial sites

The mean IL-1β, SP, and PGE2 levels, expressed as total amount per 20 s sample, in the mesial sites of experimental and control sites (E-M and C-M, respectively), are shown in Table 2. Significant differences were observed after the insertion of separators: the mean IL-1β, SP, and PGE2 values were significantly higher in the E-M sites compared with the C-M sites, at all occasions after the insertion of separators (1 min, 1 h, 1 d, 7 d).

GCF IL-1β, SP, and PGE2 levels in distal sites

The mean IL-1β, SP, and PGE2 levels, expressed as total amount per 20 s sample, in the distal sites of experimental and control sites (E-D and C-D, respectively), are shown in Table 3. IL-1β levels were significantly higher in the E-D sites compared with the C-D sites, at 1 h, 1 d,
Table 3

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th></th>
<th></th>
<th>SP</th>
<th></th>
<th></th>
<th></th>
<th>PGE2</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-D</td>
<td>C-D</td>
<td>P-value</td>
<td>E-D</td>
<td>C-D</td>
<td>P-value</td>
<td>E-D</td>
<td>C-D</td>
<td>P-value</td>
<td></td>
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<tr>
<td>−7 d</td>
<td>4.3 ± 2.1</td>
<td>4.3 ± 1.6</td>
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<tr>
<td>0 d</td>
<td>4.4 ± 2.0</td>
<td>4.2 ± 1.8</td>
<td>0.520</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>0.491</td>
<td>5.3 ± 1.0</td>
<td>5.1 ± 1.1</td>
<td>0.068</td>
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<tr>
<td>1 min</td>
<td>4.7 ± 2.2</td>
<td>4.2 ± 1.6</td>
<td>0.073</td>
<td>2.6 ± 0.7</td>
<td>2.4 ± 0.9</td>
<td>0.140</td>
<td>5.5 ± 1.1</td>
<td>5.1 ± 1.0</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>5.7 ± 3.2</td>
<td>4.4 ± 1.7</td>
<td>&lt; 0.001</td>
<td>2.9 ± 0.8</td>
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<tr>
<td>1 d</td>
<td>9.5 ± 3.2</td>
<td>4.4 ± 1.7</td>
<td>&lt; 0.001</td>
<td>5.3 ± 1.4</td>
<td>2.7 ± 0.9</td>
<td>&lt; 0.001</td>
<td>9.7 ± 2.3</td>
<td>4.8 ± 0.9</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>7 d</td>
<td>5.7 ± 2.3</td>
<td>4.2 ± 1.5</td>
<td>&lt; 0.001</td>
<td>3.4 ± 1.1</td>
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<td>0.005</td>
<td>6.2 ± 1.3</td>
<td>5.2 ± 1.1</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

Results are expressed as pg per 20 s sample.

![Box plots](image)

Fig. 2. Box plots based on the median, quartiles, and extreme values for interleukin-1β (IL-1β), substance P (SP), and prostaglandin E2 (PGE2) levels in mesial (E-M) and distal (E-D) sites of the experimental teeth at baseline, and at 1 min, 1 h, 1 d, and 7 d after the insertion of orthodontic separators. The box represents the interquartile range, which contains 50% of the values. The lines extending from the box indicate the highest and lowest values, excluding outliers. The black line across the box indicates the median. *, ** outliers. The differences (paired t-test) between mesial and distal sites are shown.

and 7 d after the insertion of separators. For the same sites, SP was significantly higher at 1 d and 7 d, whereas PGE2 was higher in the E-D sites, at all occasions after the insertion of separators (1 min, 1 h, 1 d, 7 d).

Longitudinal GCF mediator changes in mesial and distal sites

The mean percentage changes in the mesial and distal sites after the insertion of separators were assessed based on the respective baseline values (0%). The longitudinal changes in the levels of IL-1β, SP, and PGE2 in the mesial and distal sites of the experimental teeth, compared with baseline values (0 d), are shown in Fig. 2. Immediately after the insertion of separators (1 min), and at all subsequent occasions (1 h, 1 d, 7 d), a pronounced increase was observed for IL-1β in the mesial sites compared with baseline values. Similarly, the percentage increase of the SP and PGE2 levels in the mesial sites of the experimental teeth was significantly higher at
all occasions after the insertion of separators (1 min, 1 h, 1 d, 7 d), compared with the baseline value.

At the distal sites, IL-1β showed significant increases at 1 h, 1 d, and 7 d after the insertion of separators, whereas SP and PGE₂ showed significant increases at 1 d and 7 d as compared with baseline values.

Comparison of the mean percentage changes between the mesial and distal sites of the experimental teeth revealed a more pronounced increase in IL-1/β levels at 1 min and 7 d in the mesial sites as compared with the distal sites. At the same sites, a more pronounced increase was observed for SP at 1 min, 1 h, and 7 d, whereas for PGE₂ the percentage increase was significantly higher in the mesial sites compared with the distal sites, at all occasions after the insertion of separators (Fig. 2).

Discussion

The present study was designed to evaluate changes in the GCF composition during the early phase of orthodontic treatment, in relation to the time of treatment and the type of stress exerted on the periodontium (tension or compression). Although no significant differences were observed in the periodontal conditions between experimental and control sites, the levels of all three mediators were significantly higher at treatment sites compared with control sites, after the insertion of elastic separators. Furthermore, the levels of the biochemical markers in the experimental teeth depended upon the type of stress exerted on the periodontium (tension or compression).

During the last decades, the events taking place in the periodontium during orthodontic movement have been extensively studied. A mechanical force applied to a tooth is transmitted to the root-surrounding tissues of the periodontium and initiates remodelling activities that permit movement of the tooth through alveolar bone. The early phase of orthodontic tooth movement involves an acute inflammatory response, characterized by periodontal vasodilation and the migration of leukocytes out of periodontal ligament capillaries. Inflammatory mediators may trigger the biological processes associated with alveolar bone resorption and apposition. Cytokines secreted by leukocytes interact either directly with bone cells or indirectly via neighboring cells, such as monocyes, lymphocytes, and fibroblasts. The release of such molecules at the site of inflammation may alter the normal bone remodelling process, resulting in pathologic bone resorption and/or bone formation. This process will, in turn, lead to movement of the tooth (17). Modern methods of cellular and molecular biology have focused on the pivotal role played by the PDL cells in bone remodelling and repair (18–21). These studies have emphasized that PDL cells undergo osteoblastic differentiation in response to various stimuli. Even a short-term mechanical stimulus is able to induce, in the first instance, the differentiation of PDL cells towards osteoblasts, in proportions depending upon whether it is tension or pressure area (22). Furthermore, it has been shown that bone remodelling, as a result of orthodontic tooth movement, is a complex process, involving a bone-deposition phase during the early stage and a synchronous resorption and deposition phase at the later stage.

Some of the events taking place in the periodontium during orthodontic tooth movement may be studied by GCF composition analysis. In the present study, the first significant increases at the compression sites (distal sites), were obtained for IL-1/β after 1 h, and for SP and PGE₂ after 1 d. Compared with baseline values and with control sites, all three mediators thereafter remained at significantly higher levels until the end of the study (7 d). At the tension sites (mesial sites), the total amounts of all three mediators also increased significantly after the insertion of separators. These changes were already evident after 1 min, and remained at significantly higher levels, as compared with the control sites, throughout the entire study period (1 min, 1 h, 1 d, and 7 d). Our results indicate that the application of mechanical force, even a light one induced by an orthodontic separator, provokes an inflammatory reaction at both the compression and tension sites, which is reflected by changes in the GCF composition. Furthermore, the differences between the mesial and distal sites may reflect the site-specific events that take place during orthodontic treatment.

The subjects in our study showed almost identical gingival conditions for the experimental and control teeth and for the mesial and distal sites of the experimental teeth. This is mainly a result of the oral hygiene instructions given to each patient before and during treatment, and because of the short time period (7 d) during which the elastic separators were left in place. Several studies have shown that it is possible to achieve and maintain a high standard of gingival health during orthodontic treatment (23, 24). The slight deterioration of the gingival status observed after 3 months in orthodontically treated children was mainly a result of the subgingivally located orthodontic bands. In fact, banded teeth may give higher plaque index and bleeding scores than control teeth (25), especially in patients with established gingivitis (26).

As no differences in the periodontal parameters were found between experimental and control sites, and between mesial and distal sites in the experimental teeth, we may assume that plaque, BOP, and PD did not influence the production of GCF components in this study. Although the volume of GCF was not measured, we cannot rule out the possibility that the increased levels of mediators were, at least in part, associated with an increased GCF volume. SAMUELS et al. (27) reported that although no gingival health changes occur, the GCF volume can increase significantly during orthodontic treatment. Similarly, LIEB et al. (28) found that during orthodontic treatment a significant increase in GCF flow rate, unrelated to the presence of inflammation, may occur. A more recent study reported that the orthodontic force has an immediate effect on the blood vessels, resulting in higher GCF flow (29). UEMATSU et al. (12), on the contrary, were unable to show differences in GCF volume between control teeth and those subjected to orthodontic movement.
The rapid increase of all three mediators in the mesial sites of the experimental teeth (1 min) is probably a result of the mechanical irritation of the tissues induced by the placement of elastic separators. Furthermore, higher GCF levels of the biochemical mediators after the insertion of separators in the mesial sites compared with the distal sites, indicates the presence of mechanically induced inflammation. This inflammation, although not clinically recognizable, may result in an increased production of GCF mediators. IL-1β has proven to be a very sensitive indicator of inflammation, increasing significantly during experimental gingivitis before any clinically recognizable gingival changes occurred (30–32).

This molecule is thought to be secreted primarily by macrophages. However, macrophage accumulation in compressed areas has been observed at later stages, after the initiation of tooth movement. It is possible that during the initial stage of orthodontic treatment, IL-1β derives from other periodontal cell types, such as osteoclasts, as an immediate response to mechanical stress.

SP and PGE2 were also found to be associated with periodontal inflammation (8, 10) as higher levels were reported in gingivitis sites compared with healthy sites (33). Increased levels of many GCF components, such as PGE2, IL-1β, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, matrix metalloproteinase-8, etc., have been detected in teeth undergoing orthodontic tooth movement. The increased enzymatic activity in these sites, as compared with control sites, is a result of orthodontic tooth movement and is not correlated with bacterially induced inflammation (11, 34–39).

In conclusion, a simple routine procedure, such as the placement of elastic separators, may change the composition of GCF. IL-1β, SP, and PGE2 were expressed, during initial tooth movement, in sufficient amounts to be detected in the GCF. Significantly higher total amounts were detected immediately after the placement of separators; these amounts peaked after 1 d and had partially decreased 7 d later. The levels of IL-1β, SP, and PGE2 were affected by the orthodontic forces that cause bone remodeling, and their levels were significantly greater in the tension sites than in the compression sites. The increased levels of these substances did not seem to be associated with gingival inflammation, as no changes were observed clinically during the experimental trial, but seem to reflect the biologic activity that takes place during orthodontic movement.

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References


5. Pain discomfort and crevicular fluid changes induced by orthodontic elastic separators in children

C. Giannopoulou, A. Dudic, S. Kiliaridis

Pain Discomfort and Crevicular Fluid Changes Induced by Orthodontic Elastic Separators in Children

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Abstract: The objective of the present investigation was to study the experience of pain after placement of orthodontic elastic separators and the possible associations with the gingival crevicular fluid (GCF) composition changes at the level of interleukin 1-beta (IL-1β), substance P (SP), and prostaglandin-E2 (PGE2). Eighteen children (mean age 10.8 yrs) in the beginning of the orthodontic treatment were included. Molar elastic separators were inserted mesially to 2 first upper or lower molars. One of the antagonist molars served as control. The GCF was collected from the distobuccal and distopalatal sites from each molar, before (day −7, day 0) and after the placement of separators (1 h, day 1, and day 7). Pain intensity was recorded using a visual analog scale (VAS). The contents of IL-1β, SP, and PGE2 were determined by enzyme-linked immunosorbent assay. Pain intensity increased after 1 h (VAS = 11) and remained high on day 1 (VAS = 13). On day 7, no significant pain was reported. After 1 h, 1 day, and 7 days, mean GCF IL-1β levels were significantly elevated at treatment teeth compared to control teeth (highest day 1). The GCF levels of SP and PGE2 for the treatment teeth were significantly higher at day 1 and day 7 than the control teeth. All 3 mediators remained at baseline levels throughout the experiment for the control teeth. The intensity of pain at 1 h was associated to PGE2 levels (R² = 0.38; P < .05), whereas at day 1, the intensity of pain was associated to IL-1β levels (R² = 0.63, P < .0001). Thus, we report a rapid release of biochemical markers (1 h) that peaked after 1 day and partially decreased 7 days later. The intensity of pain followed a similar pattern. Associations were found between the intensity of pain and the GCF mediator levels.

Perspective: The study may help to detect, in an initial stage, individuals prone to perceive higher level of pain during orthodontic treatment. This may help in the development of methods that will better control and/or alleviate the discomfort of pain during tooth movement.

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Key words: Orthodontic movement, children, crevicular fluid, interleukin 1-beta, substance P, prostaglandin E2.

Pain associated with dental care, is a subjective reaction strongly influenced by factors such as gender, personality, and, especially, previous general and dental experience. 1,2 Until recently, little was known about pain experience during orthodontic treatment. Clinical experience shows that most patients report pain and discomfort during the first day or first couple of days of treatment and that pain intensity falls to normal levels after 7 days. 3, 5, 8, 12 Bergius et al 12 using a “model treatment” with elastic separators, reported that the experience of pain varied substantially among the subjects. The intensity of pain reached the highest levels the day after placement of separators and gradually reduced after 1 week.

In the last decades, several studies have focused on the composition of gingival crevicular fluid (GCF) and the changes that occur during orthodontic tooth movement. 6, 10, 15 Gingival crevicular fluid component analysis is a noninvasive method for studying the cellular response of the underlying periodontal ligament (PDL) during orthodontic treatment. 27 A wide variety of substances
involved in bone remodeling and produced by the PDL cells in sufficient quantities to diffuse into the GCF have already been studied. The mechanism of bone remodeling during orthodontic treatment is related on the one hand to the release of inflammatory mediators, such as prostaglandin-\(E_2\) (PGE\(_2\)) and interleukin 1-beta (IL-1\(\beta\)), and on the other hand to the production of neuropeptides, such as substance P (SP). Interleukin-1\(\beta\), a known potent cytokine produced mainly by activated monocytes, participates in the initiation of bone resorption\(^{13,16}\) either by activating osteoclasts or by stimulating the synthesis of PGE\(_2\).\(^{5,20,26,31}\) Substance P is a multifunctional neuropeptide able to stimulate bone resorption activity of osteoclasts and to modulate emotional stress. Substance P levels in GCF have been found to be significantly higher from sites showing periodontal inflammation\(^{10}\) as well as from painful teeth.\(^1\) Since each of these 3 substances (IL-1\(\beta\), SP, and PGE\(_2\)) has been related separately to pain,\(^{14,22,25}\) it would be tempting to know whether their presence in different levels in GCF could explain the finding that the experience of pain varied substantially among subjects wearing elastic separators.\(^2\)

Thus the aim of the present study was: 1) to evaluate the experience of pain perceived by the patients during a common orthodontic treatment procedure, such as the placement of orthodontic elastic separators; 2) to study the effect of this procedure on IL-1\(\beta\), SP, and PGE\(_2\) levels in GCF; and 3) to find possible associations between the levels of these substances in GCF and the perceived intensity of pain.

### Material and Methods

**Subjects**

Eighteen young patients (8 females and 10 males) were selected among patients attending the Orthodontic Clinic in Geneva for orthodontic treatment. The mean age of the patients was 10.8 years with a range of 8.9-13.8 years. The patients had to meet the following criteria: 1) good general health, 2) lack of antibiotic therapy within the last 6 months, 3) no use of anti-inflammatory drugs in the month preceding the study, 4) healthy periodontium with generalized probing depth of \(\leq 3\) mm and no radiographic evidence of bone loss, 5) crowding in one or both jaws, 6) scheduled to begin comprehensive orthodontic treatment, and 7) no eruption of the second molars. Informed consent in written form was obtained from the patients and the parents before the beginning of the study. The protocol was approved by
Table 2. VAS Assessments of General and Dental Pains (Mean, Median, and Range) and Score for Corah’s Dental Anxiety Scale

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>MEAN</th>
<th>MEDIAN</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>General pains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutting one’s finger</td>
<td>18</td>
<td>39</td>
<td>26</td>
<td>11-97</td>
</tr>
<tr>
<td>Being stung by nettle</td>
<td>17</td>
<td>47</td>
<td>49</td>
<td>9-95</td>
</tr>
<tr>
<td>Removing a splinter</td>
<td>18</td>
<td>38</td>
<td>37</td>
<td>3-96</td>
</tr>
<tr>
<td>Spraying one’s ankle</td>
<td>12</td>
<td>48</td>
<td>44.5</td>
<td>9-93</td>
</tr>
<tr>
<td>Vaccination (injection)</td>
<td>18</td>
<td>41</td>
<td>46</td>
<td>5-93</td>
</tr>
<tr>
<td>Dental pains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polishing</td>
<td>15</td>
<td>11</td>
<td>5</td>
<td>2-58</td>
</tr>
<tr>
<td>Injection</td>
<td>9</td>
<td>33</td>
<td>39</td>
<td>4-61</td>
</tr>
<tr>
<td>Drilling</td>
<td>9</td>
<td>41</td>
<td>47</td>
<td>4-74</td>
</tr>
<tr>
<td>Examination with explorer</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>0-37</td>
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<tr>
<td>Scaling</td>
<td>14</td>
<td>19</td>
<td>8</td>
<td>3-51</td>
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<tr>
<td>Corah’s Dental Anxiety Scale</td>
<td>18</td>
<td>7.5</td>
<td>7.5</td>
<td>4-12</td>
</tr>
</tbody>
</table>

Evaluation of Discomfort or Pain

Discomfort or pain was evaluated using a VAS as described above. This took place after the periodontal examination and GCF sampling procedures. Subjects were asked to report any medication (analgesics) during the study period.

GCF Sampling

Gingival crevicular fluid was collected by means of Durapore filter membranes (pore size 0.22 μm; Millipore, Bedford, MA) from the distobuccal and the distopalatal sites of the 2 experimental molars and the control molar. The collection was performed using a previously established standardized method. Briefly, after isolation of the sites from saliva, a Durapore strip was inserted 1 mm into the sulcus and left in place for 20 s. The strip was placed in a microcentrifuge tube and immediately frozen at -70°C until the day of the analysis.

Analysis of GCF Mediators Production

The content of IL-1β, SP, and PGE2 was measured in each of the 6 sites collected from each patient. A total of 540 samples were analyzed. On the day of the analysis, 350 μL phosphate buffered saline (pH 7.2) was added to the tubes containing the strips. The strips were gently shaken for 1 min and then centrifuged at 2000g for 5 min, with the strips kept at the collar of the tube to elute GCF components completely. After strip removal, the supernatant was divided into 3 aliquots for the determination of each biochemical compound. The amount of IL-1β, SP, and PGE2 was determined by enzyme-linked immunosorbent assays specific for each compound (Ruwag Diagnostics, Zurich, Switzerland; and Alexis Corporation, Laufen, Switzerland). The assays were carried out in accordance with the manufacturer’s instructions. The levels of the biochemical compounds were re-

**Mean intensity of pain (VAS)**

Figure 2: Mean VAS scores and standard error before and after insertion of orthodontic separators (n = 18).
ported as total amount (pg) per 20 s sample. This is in accordance with the suggestion of others\textsuperscript{11,17,34} who used total amounts rather than concentrations of biochemical parameters because of inherent problems of accurate determination of GCF volume. Normally, prior to sampling, saliva and supragingival plaque are removed. However, contamination with these fluids is possible, particularly in inaccessible sites (eg, the mandibular molars). Small errors in volume determination can lead to 50% or greater errors in calculation of concentration, especially when the fluid volume is small but a measurable amount of a biochemical compound is detected.

**Statistical Analysis**

For each individual, the means of the 4 experimental sites and of the 2 control sites were used to create the experimental and control groups, respectively. The paired t test was used to test the hypothesis that no difference exists between the experimental and control groups in each of the 5 sampling occasions. Longitudinal changes for each biochemical parameter were expressed as percentage of a baseline value obtained from the mean of day 7 and day 0. The paired t test was also used to compare the percentage changes between the measures of the experimental teeth at 1 h, 1 day, and 7 days.

Multiple linear regression analysis was performed to reveal correlations between the GCF component levels and pain intensity and also between pain intensity, previously experienced general and dental pain, and level of dental anxiety.

The statistical analysis was processed with SPSS for Windows (Release 13.0.0, standard version; SPSS, Chicago, IL).

**Methodologic Error and Intraindividual Variation**

The measurements of 1 site in 18 subjects from the first and second occasion (day 7 and day 0) were used to evaluate the systematic error and the intraindividual variation by paired t test (Table 1). The coefficients of variation (for clinical parameters) and the coefficient of reproducibility (for biochemical parameters) were calculated to test the methodologic error not excluding the interference of the intraindividual variation. The findings indicated that the reproducibility between these time points was sufficient to evaluate changes occurring during the experimental trial.

**Results**

**Questionnaire**

The previously experienced general pain as well as the level of dental anxiety as described in the questionnaire-based interview (Table 2) showed that the strongest previously experienced general pain was "being stung by nettles," followed by "vaccination." However, great variation was observed between subjects, the scores varying from 3 to 97 in the VAS scale. The strongest previously experienced dental pain reported by the subjects was "drilling" and "injection," though only half of the group had the experience of such a situation. Again, great variation was reported, varying from 0 to 74 in the VAS scale. Finally, the level of dental anxiety gave a median value of 2, corresponding to "being slightly nervous" before any dental treatment starts.

**Clinical Status**

All 18 participants maintained good oral hygiene throughout the study. No significant change in PI, GI, PPD, or BOP was found at any time. Both control and experimental sites showed good periodontal status, with PI and GI scores ranging between 0 and 1 and PPD of 3 mm or less.

**Evaluation of Discomfort and Pain**

The mean VAS values of the 2 occasions before the placement of the separators were 5 and 7. These values increased significantly 1 h and 24 h after the insertion of separators and returned to initial values after 7 days (Fig 2). No subject used analgesics during the study.

No correlation between pain intensity during the study

**Table 3. Mean Values for IL-1β, SP, and PGE_2 and Differences (Paired t Test) Between Experimental (E) and Control (C) Teeth**

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>SP</th>
<th>PGE_2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>Day −7</td>
<td>3.88 ± 2.29</td>
<td>3.91 ± 1.74</td>
<td>2.18 ± 0.63</td>
</tr>
<tr>
<td>Day 0</td>
<td>3.85 ± 2.10</td>
<td>3.85 ± 1.75</td>
<td>2.27 ± 0.72</td>
</tr>
<tr>
<td>1 Hour</td>
<td>8.01 ± 2.24</td>
<td>3.91 ± 1.71</td>
<td>2.80 ± 0.74</td>
</tr>
<tr>
<td>Day 1</td>
<td>8.86 ± 3.05</td>
<td>3.94 ± 1.77</td>
<td>5.22 ± 1.39</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.47 ± 2.22</td>
<td>3.97 ± 1.57</td>
<td>3.43 ± 1.03</td>
</tr>
</tbody>
</table>

Abbreviations: ns, not significant (P > 0.05); IL-1β, interleukin 1 beta; SP, substance P; PGE_2, prostaglandin E_2.

**Note:** Results are expressed as pg/20 s sample.

*P < 0.05
**P < 0.01
***P < 0.001
Figure 3. Boxplots based on the median, quartiles, and extreme values for IL-1β in experimental and control sites at baseline, and 1 h, day 1, and day 7 after insertion of orthodontic separators. Each box represents the interquartile range, containing 50% of the values. The lines extending from the box indicate the highest and lowest values, excluding outliers. The black line across the box indicates the median. Asterisks and open circles represent outliers.
and the previously experienced general and dental pain or the level of dental anxiety could be found.

**GCF Mediators**

Before the placement of separators (day −7, day 0) no significant differences of any of the 3 biochemical parameters were observed between experimental and control sites.

After insertion of orthodontic separators, significant differences were demonstrated between control and experimental teeth for IL-1β, SP, and PGE2 levels. As shown in Table 3, after the placement of separators the mean IL-1β values of the experimental teeth were significantly higher than the control teeth (1 h, day 1, day 7). The mean SP values of the experimental teeth showed no statistically significant differences 1 h after the placement of separators but were significantly higher at day 1 and at day 7. Similarly for PGE2, no statistically significant differences were found after 1 h between the experimental and control teeth, but significant differences were noted at day 1 and at day 7. At control sites, IL-1β, SP, and PGE2 did not change significantly over time.

The longitudinal changes in the level of the mediators revealed that after 1 h, IL-1β was the only marker in the experimental teeth that showed significant rapid percentage increase (P < .001). No statistically significant percentage increase was found for SP and PGE2 (Figs 3–5). After 1 day, all 3 tested substances increased to the highest percentage levels of all occasions (P < .001). At day 7, the percentage values of the experimental teeth for all 3 mediators partially decreased but still remained on a significantly higher level than the initial values (IL-1β; P < .01, SP and PGE2; P < .001).

**Association Between Discomfort or Pain and Biochemical Markers**

The initial intensity of pain (1 h) was associated to PGE2 levels (adjusted R² = 0.38; P < .05) (Table 4). At day 1, a clear association was found between IL-1β levels and the intensity of pain (adjusted R² = 0.63; P < .0001). No associations were found between the markers and the intensity of pain at day 7.

**Discussion**

The present study has shown that initial orthodontic tooth displacement by the use of separators induces pain and a rapid release of biochemical mediators. Levels of PGE2 were associated with initial pain intensity at 1 h, and IL-1β seems to be related to pain intensity after 1 day.

Our results concerning the perception of pain are in agreement with the findings of Bergius et al. who, using a similar model, observed that the highest intensity of pain was reached the day after the separators were inserted, although in a level much higher than in our study. The stronger pain reaction in that study could be due to the application of 2 separators mesially and distally to each molar, in contrast to mesially placed separators in our study, in children with the second molar not yet erupted. However, age and cultural differences between the samples could not be excluded as a reason contributing to these differences. Another difference between our results and those reported earlier by Bergius et al. is that we found higher VAS scores at the occasions before the placement of separators, which may be due to the discomfort during periodontal examination and/or GCF sampling.

The application of mechanical forces, such as those created after the insertion of elastic separators, displaces the tooth and induces an inflammatory reaction by the compression of PDL. As a result, a wide variety of substances are produced within the periodontal space that diffuse into the GCF and reflect the biologic events that take place. Several in vivo studies have used the analysis of GCF to monitor changes in a single site during orthodontic tooth movement for a certain period. In our study, after the placement of separators, significant increases in the total amount of IL-1β were observed. These increases were evident already after 1 h and 24 h. After 7 days, they decreased but did not reach baseline values. The production of PGE2 peaked later than IL-1β (at 24 h and to a lesser extent day 7), suggesting that IL-1β may have a stimulatory effect on PGE2. This is in agreement with the study of Grieve et al. who observed a peak in the production of IL-1β at 1 h and 24 h after
placement of orthodontic brackets in teeth undergoing buccal/labial tipping. Similarly, the production of PGE2 was delayed and reached a peak after 24 h. Similar results were observed by Lee et al when applying a light continuous force.

In our study, a peak production was observed for SP in the experimental sites after 1 day of the insertion of orthodontic molar separators. This is possibly due to the strong correlation of SP with PGE2, proposed as an indicator of periodontal inflammation by Hanioka et al. Besides, it can not be excluded that the stimulation of peripheral nerve terminals by means of orthodontic forces, induces the peripheral release of SP, which may act as the initial trigger for a biochemical cascade which comprises the activation of various types of PDL cells.

In the present study, associations were found between pain intensity and biochemical markers PGE2 and IL-1β levels at 1 h and 1 day, respectively, after the placement of separators. Recently, evidence has emerged that cytokines link the immune and the nervous system and may be involved in the generation of pain and hyperalgesia. Among these, IL-1β has been shown to play a major role in the generation of mechanical hyperalgesia by enhancing nociception at peripheral inflammatory tissues.

Conclusions

A simple routine procedure, such as placement of elastomer separators, may cause pain in children. The experience of pain peaked 1 day after the start of the treatment and reduced to normal level 1 week after. Interleukin-1β, SP, and PGE2 were expressed during initial tooth movement in sufficient amounts to be detected in GCF. The initial intensity of pain (at 1 h) was associated with PGE2 levels, and IL-1β was associated with pain intensity at 1 day after.

References

6. Periodontal parameters and cervical root resorption during orthodontic tooth movement

C. Giannopoulou, A. Dudic, X. Montet, S. Kiliaridis, A. Mombelli
Periodontal parameters and cervical root resorption during orthodontic tooth movement


Abstract
Objectives: To assess the relationship between periodontal parameters and cervical root resorption in orthodontically moved teeth.

Material and Methods: In a standardized experimental tooth movement in 16 periodontally healthy subjects, 29 pre-molars were tipped buccally for 8 weeks. Eighteen contralateral pre-molars not subjected to orthodontic movement served as controls. Plaque Index (PI), Gingival Index (GI), probing depth and bleeding on probing were assessed three times before and six times during the experimental phase. Teeth were extracted and scanned in a micro-computed tomography scanner. The presence or absence, and the severity of cervical root resorption were evaluated on the three-dimensional reconstruction of the scans by two calibrated examiners.

Results: Overall, periodontal parameters were not different between the test and the control teeth. Clear signs of buccal cervical resorption were detected on 27 of 29 orthodontically moved teeth and on one control tooth. Ten subjects had perfect oral hygiene and no gingivitis, whereas six subjects showed a moderate level of plaque and gingivitis (>20% occurrences of PI or GI with >0). No relationship could be demonstrated between resorption and periodontal parameters.

Conclusions: Nearly all orthodontically moved teeth showed signs of cervical resorption. Periodontal parameters were unrelated to this important side effect of orthodontic treatment.

It has been shown that orthodontic forces represent a physical agent capable of inducing an inflammatory reaction in the periodontium. This reaction is necessary for orthodontic tooth movement. The different phases of tooth movement involving the recruitment of different cells such as osteoclast and osteoblast progenitors as well as inflammatory cells have been extensively investigated (for a review, see Krishnan & Davydovitch 2006). However, the effect of orthodontic movement on the gingiva has been studied to a lesser extent. Unlike bone and periodontal ligament, gingival tissue is not resorbed after orthodontic treatment but is compressed and consequently retracted. The fact that orthodontic force does not induce gingival resorption prevents the formation of periodontal pockets and the detachment of the tooth from the gingiva. However, orthodontic treatment produces a local change in the oral ecosystem, with changes in the composition of bacterial plaque and consequently the development of gingivitis (Huser et al. 1990, Alexander 1991, Paolantonio et al. 1999).

Root resorption is an undesirable side effect of orthodontic treatment. When a tooth is tipped buccally, pressure is created on the cervical part of the buccal side of the tooth and on the apical part of the palatal/lingual side. These pressure areas are the ones where root resorption is mostly expected (Hollender et al. 1980, Linge & Linge 1991, Davydovitch 1996, Kurok et al. 1996). While apical root resorptions can be seen in radiographs (McFadden et al. 1989, Mirabella & Arun 1995a, b), to diagnose root resorption on the buccal and palatal surfaces of the root, the radiograph is an inadequate tool (Andreasen et al. 1987, Chapnick 1989). Therefore, the inflammatory changes underlying the

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Key words: cervical root resorption; orthodontic movement; periodontal parameters

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remodelling processes necessary for tooth movement have to be investigated in a wider context.

This study explores one aspect of this phenomenon by assessing the potential relationship between clinical periodontal parameters obtained during orthodontically induced tooth movement and cervical root resorption observed after treatment.

Material and Methods

Subjects

Sixteen patients (12 females and four males) were selected among patients attending the Orthodontic Department in Geneva for orthodontic treatment. The mean age of the patients was 17.7 years with a range of 11.3–43.0 years. The patients had to meet the following criteria: (i) good general health, (ii) healthy periodontium, i.e., probing depth ≤ 3 mm and no radiographic evidence of bone loss, (iii) no radiographic evidence of idiopathic root resorption, (iv) no previous orthodontic treatment, (v) no history of previous dental trauma, (vi) severe crowding in both jaws and (vii) scheduled to begin orthodontic treatment comprising extractions of at least two or four first or second premolars. Informed consent in written form was obtained from the patients (and the parents) before the beginning of the study. The protocol was approved by the Medical Ethics Committee of the University of Geneva.

Clinical examination

Before the beginning of the study, subjects received oral hygiene instructions and all tooth surfaces were cleaned and polished. Motivation for good plaque control was given to all patients. The following clinical parameters were assessed at the first (day 0) and all following visits at the buccal, mesial, distal and palatal sites of all pre-molars scheduled for extraction: Plaque Index (PI) (Silness & Löe 1964), Gingival Index (GI) (Löe 1967), periodontal probing depth (PPD) and presence or absence of bleeding on probing (BOP).

Standardized orthodontic tooth movement

The study was divided into three phases: baseline (day 0, day 7, day 21), the early experimental period (day 28, day 35, day 49) and late experimental period (day 56, day 63, day 77). The outline of the study is shown in Fig. 1.

Each patient contributed with at least one experimental and one control pre-molar. During the baseline period, only periodontal examinations were performed. In the beginning of the early experimental period (day 21), 29 pre-molars randomly assigned to the experimental group were tipped buccally. For this movement, a sectional archwire (0.019 × 0.025 TMA,Ormco, Glendora, CA, USA) was activated buccally and attached with a ligature to the bracket of the experimental tooth in order to exert a 1N force. At day 49 (beginning of the late experimental period), the amount of force was controlled and adjusted. A transpalatal and lingual arch were placed as anchorage. Eighteen contralateral pre-molars bonded with brackets but not subjected to orthodontic tooth movement served as controls. At the end of the whole experimental period (day 77), all pre-molars were carefully extracted, placed in 4% formaldehyde solution and scanned in a micro-computed tomography (CT) scanner.

Micro-CT acquisition and reconstruction

All images were acquired on a SkyScan-1076 micro-CT (SkyScan, Aartselaar, Belgium). This system is based on a cone-beam X-ray source and a charge-coupled device camera, and allows acquiring images with resolutions ranging from 35 to 9 µm. All scans were acquired at a 9 µm resolution using the following parameters: 65 kV anode voltage, 100 µA, 0.45° rotation step and 589 ms exposure time per view. For each mode, a 0.5 mm aluminium filter was installed in the beam path to cut off the softer X-rays in order to increase the accuracy of the beam-hardening correction (BHC). These settings allowed to scan a tooth in 90 min.

Cross-sectional images were reconstructed using a classical Feldkamp cone-beam algorithm (Feldkamp et al. 1984). The corrected image data were calibrated in the conventional linear scale of CT number, known as Hounsfield units (HU), defined so that water and air have values of 0 and −1000 HU, respectively.

The three-dimensional (3D) reconstructions of the micro-CT scans were analysed by two calibrated examiners (A. D. and C. G.). The amount of root resorption on the cervical part of the buccal side was assessed semi-quantitatively on randomly sequenced movies and categorized into three groups: (i) those without any resorption, including not more than one barely visible shallow resorption up to an 80 µm depth, (ii) those with moderate resorption, including a few small and shallow craters of 80–120 µm depth and (iii) those with severe resorption, including several large and deep craters of > 120 µm depth (Fig. 2).

Statistics

The following subject-based parameters were generated separately for the experimental teeth and control teeth for the three baseline examinations, the

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Early experimental period</th>
<th>Late experimental period</th>
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<tr>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 21</td>
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<td>Oral hygiene instructions</td>
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<td>x</td>
</tr>
<tr>
<td>Periodontal examination</td>
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<td>x</td>
</tr>
<tr>
<td>Extractions</td>
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<td>x</td>
</tr>
</tbody>
</table>

Fig. 1. Outline of the study.
three examinations in the early experimental period and the three examinations of the late experimental period: the percentage of sites per subject with a PI > 0, the percentage of sites per subject with a GI > 0 and the percentage of sites per subject with BOP > 0. In addition, the mean PPD (± standard deviation) was calculated for each subject in each experimental phase by summing the scores and dividing by the number of sites graded. The same parameters were assessed separately for the buccal sites.

The differences between test and control teeth were determined for each experimental period using the Fisher exact test for PI, GI and BOP and the t-test for PPD. The $x^2$ test was used to study the relationship between the patient’s hygiene level and the degree of cervical root resorption. $p$ values <0.05 were accepted for statistical significance.

The relationship between resorption and clinical parameters was further tested using logistic regression, with the presence or absence of resorption as the dependent variable and treatment or no treatment and the clinical parameters in the three experimental phases as independent variables.

The statistical analysis was performed using SPSS for Windows (Release 13.0.0., standard version, SPSS Inc., Chicago, IL, USA).

**Interrater agreement**

The interrater agreement on the micro-CT scans was assessed using Cohen’s $\kappa$. The frequencies for the presence or absence of root resorption on the cervical part of the buccal side of the root assessed by both observers are shown in Table 1. The bold figures along the diagonal show the observed frequencies of agreement when evaluating root resorption on the 3D micro-CT scan images; the corresponding expected frequencies are shown just below. The calculated Cohen’s $\kappa$ is 0.77. There appears to be a substantial agreement between the two observers in the coding of cervical root resorption on the 3D micro-CT scans.

**Results**

Table 2 shows the mean values of probing depth, the percentage of buccal sites with PI and GI scores > 0 and the percentage of buccal sites that bled upon probing for the experimental and control teeth at baseline (day 0, day 7 and day 21), the early experimental period (day 28, day 35 and day 49) and during the late experimental period (day 56, day 63 and day 77). No significant differences were observed for any of the clinical parameters between experimental and control teeth throughout the study; both showed relatively good periodontal status with a mean PPD below 3 mm, a percentage of scores of PI and GI > 0 not exceeding 26.6% and 19.8%, respectively, and no more than 10% sites with a positive BOP. During the late experimental period, an increase in the number of buccal sites with plaque and/or gingival inflammation was observed for both experimental and control teeth. However, the differences from the baseline and/or the early experimental period were not significant.

Figure 3 shows the presence or absence and the severity of cervical root resorption in the experimental and control teeth evaluated in the 3-D reconstructions of the micro-CT scans. The presence or absence and the severity of apical root resorption are reported in a companion paper. As explained under...
Table 2. Percentage of buccal sites with PI and GI > 0, BOP positive and PPD (mean ± SD) in experimental (n = 29) and control teeth (n = 18)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Early experimental period</th>
<th>Late experimental period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>experimental</td>
<td>control</td>
<td>experimental</td>
</tr>
<tr>
<td>PI (&gt; 0)</td>
<td>17.7%</td>
<td>17.5%</td>
<td>NS</td>
</tr>
<tr>
<td>GI (&gt; 0)</td>
<td>8.0%</td>
<td>9.1%</td>
<td>NS</td>
</tr>
<tr>
<td>BOP (positive)</td>
<td>3.2%</td>
<td>10.0%</td>
<td>NS</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>2.20 ± 0.31</td>
<td>2.05 ± 0.27</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant (p > 0.05); PI, Plaque Index; GI, Gingival Index; BOP, bleeding on probing; PPD, pocket probing depth; SD, standard deviation.

Fig. 3. Severity of cervical root resorption on the reconstructed micro-computed tomography images.

Table 3. Relationship between degree of cervical resorption and patients’ hygiene level

<table>
<thead>
<tr>
<th>Hygiene level</th>
<th>Buccal cervical resorption on reconstructed micro-computed tomography (CT) images</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>severe resorption</td>
</tr>
<tr>
<td>Moderate</td>
<td>11 (39%)</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>17 (61%)</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
</tr>
</tbody>
</table>

"Material and Methods", the amount of root resorption was categorized into three groups: no resorption, moderate resorption and severe resorption. Clear signs of buccal cervical resorption were detected in 27 out of 29 (93%) of the experimental teeth and one out of 18 (5%) of the control teeth. Moderate resorption was observed in one premolar of the experimental group (3.4%) and in six pre-molars (33%) of the control group.

Finally, we assessed whether the degree of cervical root resorption is correlated to the patient’s level of oral hygiene. Ten subjects had perfect oral hygiene and no signs of gingivitis, whereas six subjects showed a moderate level of plaque and gingivitis (>20% occurrences of PI and GI). However, no relationship could be demonstrated between resorption and periodontal parameters (Table 3). Logistic regression analysis confirmed these findings as treatment or no treatment was the only significant predictor for the presence or absence of resorption; the clinical parameter had no further significant effect.

Discussion

The present study was designed to evaluate the relationship between periodontal parameters and cervical root resorption in orthodontically moved teeth. Signs of buccal cervical resorption were obvious on almost all orthodontically moved teeth, as evaluated in the reconstructed images of the micro-CT scanner. However, as no significant differences were observed for any of the periodontal parameters between experimental and control teeth, it was concluded that periodontal parameters are unrelated to cervical root resorption.

Cellular and tissue reactions start immediately after force application; the early phase of orthodontic tooth movement involves an acute inflammatory response characterized by periodontal vasodilatation and migration of leucocytes out of the capillaries. Inflammation is essential to tooth movement, resulting, however, in several undesirable side effects. These effects include mainly the development of gingivitis, root resorption, gingival recession, caries, marginal bone loss and pulpal reactions.

For many years, the extent and depth of a resorbed area on the root surface of an orthodontically moved and then extracted tooth was evaluated by histology (Owman-Moll et al. 1995, Kurol & Owman-Moll 1998). However, root resorption is a 3D phenomenon and its extent needs to be quantified with precision. The micro-CT scanner is a rapid and accurate method with high resolution, providing enhanced visual and perspective assessment of root surfaces, thus offering a 3D analysis of the crevices. Thus, this method may serve as a gold standard. Hence, due to the high precision we may obtain, histology would not offer any further information.

Concerning the gingival response to tooth movement, wide variations in the clinical appearance have been described, depending on the type of tooth movement (rotation, labial movement), the force, the patient’s hygiene level, etc. (for a review, see Redlich et al. 1999). Gingivitis has been described as the most common side effect of orthodontic movement due to plaque accumulation. Several studies have shown that fixed orthodontic therapy is almost always related to inflammation of gingival tissues (Zachrisson & Zachrisson 1972, Klokkevold & Pfeifer 1974, Huser et al. 1990, Alexander 1991). This situation is mostly related to hampered oral hygiene and concomitantly to the accumulation of bacterial plaque. Furthermore, the composition of bacterial plaque following placement of orthodontic appliances changes to a more pathogenic flora (Diamanti-Kipioti et al. 1987, Paolantonio et al. 1996). An orthodontic appliance modifies locally the supragingival environment, thus affecting the colonization and occurrence of specific microorganisms. For example, Paolantonio et al. (1996)
Cervical root resorption and periodontal parameters

reported a remarkable frequency of detection of *Actinobacillus actinomycetemcomitans* (A.a) in young individuals wearing orthodontic appliances. The presence of A.a was related to the gingival bleeding index but not to the plaque levels. Other pathogenic bacteria such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Fusobacterium* have been detected after bracket placement, resulting in more gingival inflammation and bleeding (Nurkalo et al. 2006). However, these pathogens could be significantly reduced when the orthodontic appliance was removed and professional prophylaxis was given to the patient (Sallum et al. 2004).

The subjects in our study showed almost similar gingival conditions for the experimental and control teeth. This is mainly due to the oral hygiene instructions given to each patient before and during the whole experimental treatment. Several studies have shown that it is possible to achieve and maintain a high standard of gingival health during orthodontic treatment (Lundstrom & Hamp 1980a, Lundstrom et al. 1980b). The slight deterioration of the gingival status observed after 3 months in orthodontically treated children was mainly due to the sub-gingivally located orthodontic bands. In fact, banded teeth may give higher PI and bleeding scores as compared with control teeth (Huser et al. 1990), especially in patients with established gingivitis (Sallum et al. 2004).

Cervical resorption has been described as an aggressively destructive form of external root resorption, classified in the group of inflammatory resorptions and starting sub-gingivally at the cervical root surface of the tooth. Although the aetiology remains uncertain, several predisposing factors have been assessed, such as trauma, intra-oral bleeding, surgery, periodontal root scaling, bruxism and orthodontics. Heithersay (1999a) identified that all potential predisposing factors, orthodontics was the most common sole factor: when a group of 222 patients displaying varying degrees of invasive cervical resorption was analysed, orthodontics constituted 21.2% of the patients and 24% of the teeth examined. Breznitz & Wasserman (2002a, b) proposed that the term *orthodontically induced inflammatory root resorption* is more accurate to describe this pathologic consequence of orthodontic tooth movement, which should be distinct from the other type of root resorption.

Often, there are no obvious clinical signs and the condition is only detected radiographically. However, where the lesion is visible, the clinical features may vary from a small defect at the gingival margin to a pink discolouration of the tooth crown (Heithersay 1999b). Furthermore, this condition is associated with inflammation of the periodontal tissues and does not involve the pulp of the tooth. In our study, even if patients were highly motivated, cervical root resorption was present. We cannot, however, rule out the possibility that resorption would have been more severe if patients showed a poor hygiene level.

Because no differences in the periodontal parameters were found between experimental and control teeth, we may assume that plaque, BOP and PPD have no prognostic effect on cervical root resorption as a consequence of orthodontic tooth movement. Although teeth were selected to exclude any external or predisposition to resorption and were carefully extracted using forceps, resorption craters were evident in seven pre-molars of the control group (six pre-molars with moderate resorption and one pre-molar with severe resorption). This demonstrates that resorption could be a naturally occurring physiologic phenomenon. Other studies involving the identification of molecules associated with and/or responsible for root resorption during orthodontic tooth movement may be necessary.

In conclusion, cervical root resorption is a common sequela of orthodontic treatment. All the orthodontically moved teeth showed moderate and/or severe resorption on the cervical part of the buccal side. Periodontal parameters did not seem to be associated with root resorption, as no changes were observed clinically during the experimental trial.

References


**Clinical Relevance**

Scientific rationale for the study: Cervical root resorption is a common sequel of orthodontic treatment associated with inflammation of the periodontal tissues. Nevertheless, prediction and prevention are still impossible.

Principal findings: All orthodontically moved teeth showed signs of cervical resorption. However, no relationship could be demonstrated between resorption and periodontal parameters.

Practical implications: Routine periodontal parameters are unrelated to cervical root resorption resulting from orthodontic tooth movement.
7. Diagnostic accuracy of digitized periapical radiographs validated against micro-computed tomography scanning in evaluating orthodontically induced apical root resorption

A. Dudic, C. Giannopoulou, M. Martinez, X. Montet, S. Kiliaridis
Diagnostic accuracy of digitized periapical radiographs validated against micro-computed tomography scanning in evaluating orthodontically induced apical root resorption


The aim of this study was to validate the use of digitized periapical radiographs in evaluating orthodontically induced apical root resorption against micro-computed tomography (micro-CT) scanning as a criterion standard test. In a standardized experimental protocol, 29 premolars in 16 subjects were tipped buccally for 8 weeks. Nineteen contralateral premolars not subjected to orthodontic treatment served as controls. Standardized periapical radiographs were taken before and after the experimental period (Rx method). These teeth were extracted and scanned using a micro-CT technique with a 9 μm resolution. Two calibrated examiners assessed blindly the presence or absence of apical root resorption on digitized radiographs and three-dimensional reconstructions of the scans. Significant differences were detected between the orthodontically moved teeth and controls: 86% of the orthodontically moved teeth and 21% of the control teeth showed apical root resorption when using micro-CT as a validation method. A total of 55% of the experimental teeth and 5% of the control teeth showed resorption when assessed using Rx method. The Rx method showed a specificity of 78% and a sensitivity of 44%, which means that less than half of the cases with root resorption identified using a CT scanner were identified by radiography. Nearly all the orthodontically moved teeth showed apical root resorption. Apical root resorption may be underestimated when evaluated using digitized periapical radiographs.

External apical root resorption is a common, undesirable side effect of orthodontic treatment with an incidence varying from 22 to 100% (1, 2). Small areas of surface resorption can be observed histologically on the pressure side of almost all roots shortly after the application of orthodontic force (3, 4). In most cases this resorption does not decrease the functional capacity of the involved tooth (5), but in other cases the degree of resorption may become important, resulting in an unfavourable crown/root ratio and thus less periodontal support for the tooth, decreasing its longevity (6, 7).

Clinically, radiography is the only method for diagnosing root resorption. Different radiographic methods have been used, but to date the recommended tool for detecting root shortening during orthodontic treatment is periapical radiography (8, 9). This technique results in less image magnification and distortion compared with panoramic imaging, thus providing greater accuracy (10).

However, a certain degree of resorption is required before being detectable on the radiograph. Orthodontically induced root resorption after the first weeks of treatment, verified histologically, is not visible in periapical radiographs (3). Therefore, several authors have stated that conventional radiographs are not an adequate tool for accurately diagnosing the early stages of resorption (11, 12), especially when teeth are moved in a buccal direction. In order to obtain greater accuracy, digital subtraction imaging has been introduced (13-16). This method was shown to be more sensitive than conventional radiography for detecting simulated external root resorption cavities on dry human mandibles (17, 18). The use of a mathematical computer-based reconstruction of pretreatment and post-treatment periapical images was shown to be a reliable method for the measurement of apical root resorption following orthodontic movement (19). In spite of the great potential of digital

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Key words: apical root resorption; digitized periapical radiographs; micro-CT scanner; orthodontic movement

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reconstruction for more accurate analysis of root resorption, (20), the method is time consuming and not easily clinically applicable.

Owman-Moll et al. (21, 22) introduced an experimental clinical model for the evaluation of root resorption in previously moved and finally extracted premolars; root resorption was evaluated by histology and a conventional radiographic method. The same model was later used by others for the evaluation of orthodontically induced root resorption (23). Recently, the microcomputed tomography (micro-CT) technique has been shown to be a rapid and accurate method with high resolution, providing enhanced visual and perspective assessment. The method has been mainly used for visualizing and quantifying bone architecture and development (24) as well as trabecular structures (25). Micro-CT can also be used to visualize and quantify the resorption crater on the root surfaces of extracted teeth that have undergone orthodontic tooth movement (26, 27). A three-dimensional (3D) analysis of the craters can be obtained without performing histological analysis.

By using the Micro-CT scanner as criterion standard (gold standard) on extracted teeth that had previously undergone experimental orthodontic treatment, we aimed to investigate the diagnostic accuracy of digitized periapical radiographs in detecting orthodontically induced root resorption.

Material and methods

Sixteen patients (12 women and 4 men) were consecutively recruited among patients starting orthodontic treatment at our University. The mean age of the patients was 17.7 yr with a range of 11.3–43.0 yr. The patients had to meet the following criteria: (i) good general health; (ii) no previous orthodontic treatment; (iii) severe crowding in both jaws; (iv) no radiographic evidence of idiopathic root resorption; (v) a schedule to begin orthodontic treatment with the need of at least two (5 patients), or four (11 patients) first or second premolar extractions; and (vi) complete afilliation of all premolars included in the study. Informed consent in written form was obtained from the patients before the start of the study. The protocol was approved by the Medical Ethics Committee of our University.

Standardized orthodontic tooth movement

A standardized experimental tooth movement was carried out in all the patients. Each patient contributed with at least one experimental and one control premolar. Twenty-nine premolars randomly assigned to the experimental group were tipped buccally for 8 wk. For this movement a sectional archwire (0.019 x 0.025 titanium molybdenum alloy [TMA]) was activated buccally, and attached with a ligature to the bracket of the experimental tooth in order to exert an initial force of 1 N (statically determinate force system). In the middle of the experimental movement (after 4 wk), the amount of force was controlled and adjusted. A transpalatal arch and a lingual arch were placed as anchor. Nineteen contralateral premolars bonded with brackets, but not subjected to orthodontic tooth movement, served as controls.

Periapical radiographs and film evaluation

Periapical radiographs were taken before and after completion of the experimental tooth movement (immediately before the extractions) using intra-oral radiographic film (Kodak, Eastman Kodak, Rochester, NY, USA; Ultraspeed D. 30 x 40 mm, 7 mA, 70 kV, 0.20 s). In order to achieve optical projection geometry, we used the paralleling technique, in which the receptor is placed parallel to the long axis of the tooth, and the control ray of the X-ray beam is at a right angle to both (28).

Radiographs were digitized using a radiograph scanner (Epson Expression 1600 Pro, Epson-Epson Corp., Tokyo, Japan) with a 600 d.p.i. (dots per inch) resolution enhanced for contrast and calibrated for luminosity. The images were stored in the jpeg format in highest quality (2:1 compression). Mild jpeg compression does not seem to compromise the diagnostic performance in digital radiography (29). Two calibrated examiners (AD and CG) assessed separately and blindly the presence or absence of apical root resorption on the digitized radiographs on a 15” computer-screen under standardized light conditions not exceeding 50 lux. The pre-experimental and postexperimental radiographs were shown on the same slide in a randomized sequence (Fig. 1) and the examiners were additionally blinded by hiding the crowns of the teeth with a black hand strip. Any visual changes on root shape and length were regarded as ‘root resorption lesions’. In the event of disagreement a new evaluation in common was performed and this consensus was used for the final evaluation.

Micro-scanner image acquisition and reconstruction

At the end of the experimental period (8 wk), all premolars (experimental and control) were carefully extracted by the same clinician (AD), placed in 4% formaldehyde solution and scanned in a micro-CT scanner. Images were acquired on a SkyScan-1076 micro-scanner (SkyScan, Aartselaar,
Radiographs and the degree of apical root resorption on the 3D micro-scanner images. A chi-square test was used to test the hypothesis that no difference exists between the orthodontically moved and control teeth. The validity of the digitized periapical radiographs to detect apical root resorption after orthodontic tooth movement was assessed using the high-resolution 3D micro-scanner images as the criterion standard. The frequency of resorption, the sensitivity and specificity, the positive and negative predictive values, and odd ratios and their confidence intervals (CIs) were calculated. The statistical analysis was processed using spss for Windows (Release 13.0.0, standard version; SPSS, Chicago, IL, USA).

Inter-rater agreement

The inter-rater agreement on both the micro-CT scan images and periapical radiographs was assessed using Cohen's Kappa. The frequencies for the presence or absence of apical root resorption on the 3D micro-scanner images and digitized periapical radiographs assessed by both observers are shown in Tables 1 and 2, respectively. The bold figures along the diagonal show the observed frequencies of agreement when evaluating root resorption on the 3D micro-scanner images and on the digitized periapical radiograph respectively; the corresponding expected frequencies are shown just below. The calculated Cohen's Kappa was 0.78 for both methods. There appeared to be substantial agreement between the two observers in the evaluation of apical root resorption.

Results

Comparison between orthodontically moved and control teeth

The hypothesis that no difference exists between the orthodontically moved teeth and control teeth concerning apical root resorption is rejected. Figure 3 shows that significant differences were observed between the orthodontically moved teeth and the controls: when apical root resorption was evaluated in the 3D reconstructions

<table>
<thead>
<tr>
<th>First observer</th>
<th>No resorption</th>
<th>Resorption</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No resorption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>16</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Expected count</td>
<td>7.1</td>
<td>11.9</td>
<td>19.0</td>
</tr>
<tr>
<td>Resorption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>2</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Expected count</td>
<td>10.9</td>
<td>18.1</td>
<td>29.0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>39</td>
<td>48</td>
</tr>
<tr>
<td>Expected count</td>
<td>18.0</td>
<td>39.0</td>
<td>48.0</td>
</tr>
</tbody>
</table>

The values shown in bold along the diagonal represent the observed frequencies of agreement when evaluating root resorption on the 3D micro-CT scan images and on the digitized periapical radiographs, respectively.
Table 2

<table>
<thead>
<tr>
<th>Second observer</th>
<th>No resorption</th>
<th>Resorption</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>First observer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No resorption</td>
<td>27</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Expected count</td>
<td>18.1</td>
<td>12.9</td>
<td>31.0</td>
</tr>
<tr>
<td>Resorption</td>
<td>1</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Expected count</td>
<td>6.9</td>
<td>7.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>Expected count</td>
<td>28.0</td>
<td>20.0</td>
<td>48.0</td>
</tr>
</tbody>
</table>

The values shown in bold along the diagonal represent the observed frequencies of agreement when evaluating root resorption on the 3D micro-CT scan images and on the digitized periapical radiographs, respectively.

Fig. 3. Distribution, in percent, of apical root resorption in experimental and control teeth by micro-computed tomography (micro-CT) analysis and periapical radiographs. Black bars, apical root resorption; white bars, no resorption. **p < 0.01, ***p < 0.001.

of the micro-CT scans, clear signs of resorption were obvious in 25 out of 29 (86%) of the experimental teeth and in 4 out of 19 (21%) of the controls (P < 0.001). Similarly, when apical root resorption was evaluated on the digitized periapical radiographs, signs of resorption were detected in 16 out of 29 (55%) of the experimental teeth and in 1 out of 19 (5.2%) of the controls.

Diagnostic accuracy of digitized periapical radiographs compared with the micro-CT criterion standard test

Table 3 shows the number (and percentages) of teeth with absence or presence of apical root resorption that were evaluated using periapical radiographs and micro-CT scanning. The footnote contains calculations of estimates of the measures of interest. We evaluated the specificity and sensitivity of the radiographic method to detect apical root resorption: the method showed a high specificity of 78%, which means that 78% of the teeth with 'no resorption' were diagnosed correctly using digitized periapical radiographs. However, a relatively low sensitivity of 44% was obtained, which means that less than half of the teeth with apical root resorption were identified correctly using the digitized periapical radiographs. The positive predictive value of the radiography was found to be 76%, whereas the negative predictive value was 48%. The likelihood ratio for a positive test result was 2.1, which indicates that a positive result is twice as likely to occur in a tooth with resorption than in one without.

Discussion

The study aimed to determine the accuracy of digitized periapical radiographs in the diagnosis of orthodontically induced root resorption. The radiographic findings were validated against those obtained by the micro-CT scanner reconstructions of the extracted teeth: while 55% of the experimental teeth were radiographically diagnosed as presenting apical root resorption, 86% of the same teeth showed apical root resorption when analyzed using the micro-CT method.

The high incidence of apical root resorption in the experimental teeth observed using the micro-CT method confirms previous studies showing that after application of an orthodontic force, small areas of surface resorption always occur, especially on the pressure sides of the roots (22, 31–33). As expected, in our study, buccal tipping forces occurred and thus the buccal cervical and lingual apical regions had undergone tissue compression, the buccal apical and lingual cervical were under tension, and the mid-root regions had undergone a mixture of both compression and tension (34).

Digital radiography (9, 13, 35–38) was introduced in order to improve the diagnostic accuracy over that obtained using conventional radiography. However, conflicting results have been reported. Furthermore, a difference between the radiographic aspect and the
histological condition of the affected teeth exists. LAUX et al. (39) assessed the reliability of routine single radiographs in the diagnosis of inflammatory apical root resorption by correlating the radiographic and histological findings. Nineteen percent of the teeth were diagnosed radiographically as having apical inflammatory root resorption, whereas histologically, 81% of the teeth revealed apical inflammatory root resorption.

Previous studies examined root resorption craters in a two-dimensional (2D) manner by using confocal, light or scanning electron microscopy (40–42). However, root resorption is a 3D phenomenon and its extent needs to be quantified with precision. The accuracy of the 3D evaluation of root resorption craters over the 2D histological and radiographic evaluation has already been studied (23, 43). CHAN et al. (44) achieved both 3D visualization and volumetric measurement of the resorption craters. Stereo scanning electron microscopy (SEM) images were imported into a 3D red–green stereo anaglyph coding of a 3D flight simulation program in order to visualize the root resorption craters. A special software program was developed for the volumetric quantification of each individual crater. The advantage of the 3D analysis was that the craters could be visualized from different perspectives, thus enhancing the extent, topography, and morphology of the resorption craters. Furthermore, the same group evaluated the volume of root resorption craters after the application of light (25 g) and heavy (225 g) orthodontic forces. Using a very similar force application design, they found that the heavy force group had 3.31-fold greater total resorption volume than the light force group (45).

Over the last decade, micro-CT has been introduced in order to quantify complex geometries at small resolutions, even in the order of 10 μm (46). In the field of Dentistry, the method was adapted in order to receive high-resolution 3D images of extracted teeth: the root resorption craters became clearly visible, thus helping us to identify even minor root resorption spots on control teeth that had not undergone any orthodontic movement. Although teeth were selected to exclude any external or predisposition to resorption, craters were evident in 4 out of the 19 control teeth (21%), demonstrating the idiopathic nature of this phenomenon. Furthermore, we obtained four false-positive results, which means that four teeth were evaluated in the radiographs as presenting resorption craters which could not be confirmed in the micro-CT images. One explanation for this could be that two of the premolars showed curved apices, and the other two were close to the apices of the neighbouring teeth, thus making the evaluation difficult and leading to an overestimation.

In conclusion, apical root resorption is a common sequela of orthodontic treatment. The high-resolution 3D micro-CT images were used as a criterion standard to estimate the degree of this undesirable side effect of orthodontic treatment. The comparison of the radiographic and micro-CT scanner method shows the limited accuracy of periapical radiographs to detect apical root resorption.

Acknowledgments — The present work was supported by the Swiss National Science Foundation (grant no. 3200-06480.01).

References


8. Detection of apical root resorption after orthodontic treatment by using panoramic radiography and cone-beam computed tomography of super-high resolution

A. Dudic, C. Giannopoulou, M Leuzinger, S. Kiliaridis
Detected of apical root resorption after orthodontic treatment by using panoramic radiography and cone-beam computed tomography of super-high resolution

Alexander Dudic, Catherine Giannopoulou, Michael Leuzinger, and Stavros Kiliaridis
Geneva and Winterthur, Switzerland

Introduction: Apical root resorption is an adverse side effect of orthodontic treatment. We compared panoramic radiography (OPT) with cone-beam computed tomography (CBCT) in evaluating orthodontically induced apical root resorption. Methods: The study sample comprised 275 teeth in 22 patients near the end of orthodontic treatment with fixed appliances. Two calibrated examiners assessed blindly the presence or absence and the severity of apical root resorption on the OPT images after treatment and the corresponding reconstructed CBCT images. Resorption was evaluated as no, mild, moderate, severe, and extreme. Results: On the OPT images, 17 teeth (6.2%) could not be evaluated. Statistically significant differences were found between the two methods: 56.5% and 31% of the teeth showed no resorption by OPT and CBCT, respectively; 33.5% and 49% of the teeth showed mild resorption, whereas 8% and 19% showed moderate resorption by OPT and CBCT, respectively. Severe resorption was found in only 2 teeth by CBCT. Conclusions: Apical root resorption after orthodontic tooth movement is underestimated when evaluated on OPT. CBCT might be a useful complimentary diagnostic method to conventional radiography, to be applied when a decision on continuation or modification of the orthodontic treatment is necessary because of orthodontically induced root resorption. (Am J Orthod Dentofacial Orthop 2009;135:431-7)

External apical root resorption is a common undesirable side effect of orthodontic treatment. The clinical diagnosis is based mainly on routine radiographic procedures, such as panoramic (OPT) and periapical radiography. However, some root shortening is required before it is detectable on the radiograph. Furthermore, OPT has been shown to overestimate the amount of tooth loss by 20% or more compared with periapical radiography, and digitized periapical radiographs have been shown to underestimate apical root resorption compared with a micro-computed tomography scanner. Until now, there was no gold standard for the detection of orthodontically induced root resorption.

Cone-beam computed tomography (CBCT) is a new radiographic method with application in several diagnostic areas, such as implant treatment, oral surgery, endodontic treatment, and temporomandibular joint imaging. The great advantage of this technology is that it offers 3-dimensional (3D) imaging of dental structures and provides clear images of highly contrasted structures, such as bone. Compared with conventional computed tomography, CBCT technology in clinical practice has important advantages such as minimization of the radiation dose, image accuracy, rapid scan time, fewer image artefacts, chair-side image display, and real-time analysis. In orthodontics, CBCT imaging has been restricted to impacted teeth, temporomandibular joint visualization, determination of bone volume conducive to orthodontic tooth movement, and cleft patients. However, the diagnostic ability of CBCT in detecting orthodontically induced apical root resorption has not been sufficiently studied. The purpose of this study was to compare the efficacy of OPT and CBCT in the detection of apical root resorption after orthodontic tooth movement.
MATERIAL AND METHODS

Twenty-two patients (8 female, 14 male; mean age, 16.7 years; range, 12.6–17.2 years) were included in this study. They were selected from a private orthodontic practice in Winterthur, Switzerland. They were near the end of orthodontic treatment with fixed appliances and, after OPT evaluation, were further referred to study the proximity of neighboring roots with CBCT.

OPT images were acquired with the Orthopantomograph (CraneX Excel, Soredex, Tuusula, Finland) and stored in the TIF format. The CBCT images were obtained with the 3D Accuitomo (J. Morita, Kyoto, Japan). Two sizes of imaging areas (40 × 40 and 60 × 60 mm) were used with super-high resolution (2.0 line pairs per millimeter; voxel size, 0.125 mm). The plane of primary reconstruction was aligned parallel to the long axis of the examined tooth by using iDixel software (J. Morita), as suggested by the manufacturer. Depending on the region of interest, either 1 or 2 CBCT images were taken from each patient.

Two calibrated examiners (A.D. and C.G.) assessed separately and blindly the presence or absence and the degree of apical root resorption in the OPT and CBCT images using the scoring system of Levander and Malmgren that classifies it into 5 grades: 0, no root resorption; 1, mild resorption, with the root of normal length and only an irregular contour; 2, moderate resorption, with small areas of root loss and the apex having an almost straight contour; 3, severe resorption, with loss of almost one third of root length; and 4, extreme resorption, with loss of more than one third of the root length. In case of disagreement between the 2 examiners, a new evaluation was made, and this consensus was used for the final evaluation. The Cohen kappa showed substantial agreement between the 2 observers with the CBCT method (value, 0.63) and poor agreement with the OPT (value, 0.46).

Figures 1 and 2 show the grades of apical root resorption evaluated by the OPT and the CBCT, respectively. Only the grades found in our sample are shown.

The Pearson chi-square test was used to test the null hypothesis that there is no difference in evaluating apical root resorption on OPT and CBCT images. The statistical analysis was processed with SPSS software for Windows (release 13.0.0, standard version, SPSS, Chicago, Ill).

RESULTS

A total of 275 teeth were evaluated by OPT and CBCT for apical root resorption: 208 maxillary teeth and 67 mandibular teeth (92 incisors, 43 canines, 76 premolars, and 64 molars). Evaluation was impossible in 6 incisors, 4 canines, 5 premolars, and 2 molars with the OPT method. The comparison between the 2 methods was assessed in teeth evaluated by both methods; 258 teeth were assessed for statistical analysis.

The numbers and percentages of teeth with the different grades of apical root resorption as evaluated by OPT and CBCT are shown in Table I. Significant differences were observed between the 2 methods and for all grades of resorption. One hundred forty-five teeth were evaluated by OPT as having no resorption, whereas, by CBCT, only 80 teeth had no resorption; 92 teeth showed mild apical root resorption with OPT at 128 teeth with CBCT. Only 21 teeth had moderate resorption with OPT, but 48 teeth had it with CBCT. Furthermore, 2 teeth had severe resorption-grade 3—when assessed with CBCT. Overall, the differences between the 2 methods in evaluating apical root resorption were significant for both the maxilla and the mandible (P <0.001 and P <0.002, respectively); the maxillary incisors showed the most pronounced differences (Table II).
Table I. Evaluation of apical root resorption in all teeth by OPT and CBCT

<table>
<thead>
<tr>
<th>CBCT</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>62</td>
<td>18</td>
<td>0</td>
<td>80</td>
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<tr>
<td>1</td>
<td>70</td>
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<td>12</td>
<td>21</td>
<td>15</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>92</td>
<td>21</td>
<td>258</td>
</tr>
</tbody>
</table>

Table II. Evaluation of apical root resorption in maxillary incisors by OPT and CBCT

<table>
<thead>
<tr>
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<th>1</th>
<th>2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>20</td>
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<td>9</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>42</td>
<td>10</td>
<td>65</td>
</tr>
</tbody>
</table>

DISCUSSION

A gold standard for the detection of orthodontically induced root resorption is still missing. However, this was beyond the goal of our study. Our aim was to determine the accuracy of OPT in the diagnosis of apical root resorption after orthodontic treatment. We compared the OPT findings with those obtained with CBCT: whereas 69% of the teeth were diagnosed as having apical root resorption by CBCT, only 44% showed apical root resorption with OPT. Furthermore, 17 teeth could not be evaluated by OPT. Overall, these results suggest that apical root resorption might be underestimated by OPT.

Root resorption is a 3D phenomenon, and its extent must be quantified with precision. Until now, radiographic methods, although they have important limitations, are the only methods to evaluate apical root resorption. However, the results need to be interpreted with caution. Only histologic or scanning electron microscopy studies can give exact results, but these are performed on experimentally moved and then extracted premolars. By the OPT method, images of mandibular incisors proclined during treatment can be foreshortened or the apices might lie outside the focal plane, thus resulting in "shorter" teeth after treatment. Furthermore, during orthodontic treatment,
the angulations of the incisors might change, and this can affect the length of the radiographic image of the tooth; thus, the amount of root resorption is not evaluated precisely.\(^\text{19}\) Finally, the lack of reproducibility is also an important factor that limits the diagnostic accuracy of the OPT.\(^\text{20}\)

CBCT provides highly detailed 3D imaging with only 1 x-ray exposure of approximately 18 seconds. Imaging can be obtained at any angle, thus offering optimum viewing and eliminating superimpositions. CBCT images have provided reliable data on root angulation\(^\text{21}\) and the management of impacted canines.\(^\text{22}\)\(^\text{23}\)\(^\text{24}\) The diagnostic ability of CBCT to detect simulated external root resorption was studied by Silveira et al.\(^\text{25}\) Cavities of different depths and diameters were prepared on the cervical, middle, and apical thirds of the buccal surfaces. The evaluation of the CBCT’s diagnostic ability showed high sensitivity and excellent specificity; only very small cavities in the apical third were more difficult to detect compared with other cavities.

For the moment, it is evident that CBCT cannot replace OPT, which remains the primary imaging modality. However, in certain complex cases, the 3D data sets might be more suitable than conventional radiographs. Thus, if signs of moderate root resorption are visible on OPT during the initial or middle phase of orthodontic treatment, CBCT can be useful in evaluating the severity of the situation to help make the decision on continuation and possible modification of orthodontic treatment.

We found that, compared with OPT, CBCT has an advantage in detecting root resorption during orthodontic treatment but has also has a greater medical risk. Therefore, CBCT imaging should be used for 2 main reasons: in research, it might increase our knowledge of root resorption, and, in clinics, CBCT images could help to monitor patients (with syndromes, agenesis, aberrant root forms) at risk for developing severe root resorption during orthodontic tooth movement.

CONCLUSIONS

CBCT is a powerful tool to show apical root resorption during orthodontic treatment, whereas OPT underestimated it. CBCT might be a useful complementary diagnostic method to conventional radiography, to be applied when determining whether to continue or modify orthodontic treatment because of orthodontically induced root resorption.

REFERENCES


Figure 1: Placement of orthodontic elastic separators mesially to the first upper molars and GCF sampling from the mesial and distal sites of the experimental teeth.
Figure 2: Experimental orthodontic tooth movement in 2 of the 4 first premolars: the upper left and lower left premolars are tipped buccally by exerting a 1N force (experimental teeth), the upper right first premolar is free of any bracket (control), the lower right premolar is bonded with bracket but without any force application (control)
Figure 3: Panoramic radiographs of a 13 years old girl before and after orthodontic treatment. Severe crowding was treated by extraction of the four first premolars. Severe root resorption is observed in the anterior region of both jaws 2 years after the orthodontic tooth movement.
Figure 4: Presence or absence and severity of cervical root resorption in 3 premolars evaluated in the micro-CT scans: the first premolar shows no resorption, the second premolar shows moderate resorption and the third premolar shows severe resorption.
Figure 5: Presence or absence and severity of apical root resorption in 3 premolars evaluated in the micro-CT scans: the first premolar shows no resorption, the second premolar shows moderate resorption and the third premolar shows severe resorption.
Figure 6: Apical root resorption evaluated in the OPT and CBCT images. No resorption is observed for the right upper lateral incisor in the OPT, whereas severe resorption is observed in the CBCT image.
10. ON GOING RESEARCH

In the work described in this Thesis, some aspects on the physiopathologic mechanism that takes place during orthodontic movement, have been addressed. However some questions remain unsolved and new ones have arisen. These questions are subject of ongoing research within our research group, which is described below.

10.1. Amount of tooth movement and severity of root resorption

Orthodontic casts are a useful tool in orthodontics for diagnosis and treatment planning. However, the storing of casts for many years, the transportation as well as the risk of damage or loss, make that the use of orthodontic casts has significant limitations. Two dimensional digital imaging (2DI) can be used as an alternative to casts in the assessment of malocclusion and orthodontic treatment need (201, 202).

Based on our data of the standardized experimental orthodontic tooth movement, we investigated if the variation of the severity of root resorption observed at the end of the experimental period was related to the amount of tooth movement.

The plaster models obtained from all patients before and after the experimental tooth movement were digitized and superimposed, in order to evaluate the amount of tooth movement. (Figure 1) The severity and localization of root resorption on the three dimensional reconstruction of the scans were evaluated as previously described (Chapters 6 and 7). To compare the differences in tooth movement between the experimental and control group an unpaired t-test was done. Descriptive statistics was used to show the severity and localization of root resorption. The Pearson’s correlation coefficient was calculated in order to test the correlation between the severity of root resorption and the amount of tooth movement.

We found that 93 % of the orthodontically moved teeth showed severe root resorption at the buccal cervical part of the root and 78 % at the lingual apical part of the root (Figure 2). Significant differences for the amount of tooth movement were found between the orthodontically moved teeth (2.41 ± 1.07mm) and the controls (0.39 ±
0.26mm) (Figure 3). Furthermore, the severity of root resorption was correlated to the amount of tooth movement ($R^2=0.46; p<0.001$) (Figure 4).

We concluded that application of a 1 N force exerted over a 2 month period provokes severe root resorption at the compression sites and that the severity of resorption is correlated to the amount of tooth movement.

**Figure 1.** Measuring the amount of tooth movement on digitized casts

**Figure 2.** Distribution and severity of resorption sites on the reconstructed micro-CT images
Figure 3. Amount of tooth movement

Figure 4. Correlation between the amount of tooth movement and the severity of root resorption
10.2. Gingival crevicular fluid and root resorption

In our knowledge, very few studies exist on the association between GCF components' level and root resorption during orthodontic movement. Recently, the dentine phosphoproteins (DPP, non-collagenous proteins of dentin matrix) and the dentine sialoprotein (DSP) were detected in GCF: the levels of these molecules were found to be higher in primary teeth undergoing physiologic root resorption and in teeth with orthodontically induced severe root resorption as compared to non-resorbing controls (203-205). The role of cytokines of the RANKL/RANK/OPG system in inducing bone remodeling during orthodontic tooth movement has been demonstrated: higher expression of RANKL was found in the compression side, whereas higher expression of OPG was found in the tension side. Interestingly, the compressed periodontal ligament cells obtained from tissues with severe external apical root resorption may produce large amounts of RANKL (206). In GCF the expression of these molecules has been mainly studied in relation to periodontal disease (189, 190). What remains to be elucidated is whether the RANKL/OPG system may provide an important link between bone remodeling, orthodontic tooth movement and root resorption during orthodontic tooth movement.

During the experimental tooth movement described in chapters 6 and 7, the GCF was collected at the buccal/labial and palatinal/lingual sites of the first or second premolars in the 4 quadrants that were planned to be extracted at the end of the experimental period. The collection was made by means of durapore filter membranes. Briefly, after isolation of the sites from saliva, a first durapore strip was inserted 1mm into the sulcus and left in place for 15sec. After removal of the first strip and after waiting 3 min, a second durapore strip was inserted in the same site, in the same manner. The 2 strips were placed into a microcentrifuge tube and immediately frozen at –70°C until the day of the analysis. In case of visible contamination with blood, the strips were discarded. A total of 104 samples were collected in each patient, during the entire study.
We have started the analysis of four markers in the GCF samples of 30 patients who participated and completed the study:

- Receptor activator of NFkB ligand (RANKL)
- Osteoprotegerin (OPG)
- Osteopontin (OPN)
- Osteocalcin (OC)

On the day of the analysis, 60 µl of phosphate-buffered saline (PBS, pH 7.2) is added to the tubes containing the strips. The strips are gently shaken for 1 minute and then centrifuged at 2000xg for 5 minutes, with the strips kept at the collar of the tube in order to elute GCF components.

Gingival crevicular fluid samples are analyzed by the Bio-Plex 200 Suspension Array System provided by Bio-Rad. The system is a flow-based dual-laser system for simultaneously identifying and quantifying up to 100 different analytes in a single biomolecular assay (xMAP technology). The system detects and measures molecules bound to the surfaces of fluorescent microspheres, thus providing highly accurate analysis of serum, culture media and other biological samples as small as 25 µl. To perform a multiplex reading, samples are mixed with conjugated microsphere and reactant mixtures, and fluorescent reporter molecules are added. The assays are loaded into the well of a 96-well microtiter plate and the plate is inserted into the microplate platform. The platform and array read are controlled by a computer running Bio-Plex Manager software. The detection system in the array reader uses two lasers to analyze the microspheres in the system. The first laser identifies each microsphere and its associated analyte, and the second measures the amount of analyte in the sample.

The principal advantage of the system is that a large number of markers in a small sample can be quantified simultaneously. The same volume would only be sufficient for the analysis of a single marker by ELISA. This can be translated into substantial savings in the cost of reagents and time to perform the assay completely. After strip removal, the supernatant is divided into two aliquots for the determination of the biochemical compounds. One aliquot (25µl) serves for the determination of RANKL by using a single plex assay kit and the other aliquot serves for the simultaneous determination of OPG, OPN and OC by using a three-plex assay kit. Four captured
antibodies for the specific markers are coupled to specific bead sets with different fluorophores. Using multiple bead sets, 4 different assays are simultaneously performed and detected on two aliquots of each individual sample.

At present, it is not possible for the clinicians to predict root resorption with confidence, to prevent or reduce its occurrence and also to reverse the destruction of tooth structure during orthodontic treatment. This is especially important for high risk patients where severe root resorption occurring during orthodontic treatment, may result in the total loss of tooth in treatment. The results of the ongoing study may help: i) to better understand the physiopathologic mechanism that takes place during orthodontic movement, responsible of the root resorption  ii) to elucidate if one or combination of several markers in crevicular fluid could help us to identify a patient at risk for extensive root resorption during orthodontic treatment iii) to detect root resorption before the radiographic observation and eventually to avoid in the future the radiography as method for diagnostic screening of the presence of root resorption.
11. GENERAL DISCUSSION

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament and alveolar bone. The remodeling activities and the ultimately tooth displacement are the consequence of an inflammatory process. The cellular, biochemical and molecular events that take place during orthodontic tooth movement have been extensively studied. Although inflammation is essential in the remodeling activities, it may result in several undesirable side effects. Thus, not only the aesthetic and functional outcome but also the adverse events for the teeth and the supporting tissue related to the orthodontic tooth movement should be considered.

Orthodontic treatment is very often associated to gingival inflammation, as a result of the local change in the microbial ecosystem and in the composition of the bacterial plaque. Patients with good oral hygiene may develop gingival inflammation during orthodontic treatment. Mild to moderate gingivitis with bleeding on probing and gingival enlargement is evident, especially when fixed orthodontic appliances are used (45-48). Gingivitis does not progress into periodontitis, in terms of loss of attachment and bone probably due to the restriction of plaque-induced inflammatory reaction in the supra-alveolar connective tissue. According to Zachrisson and Zachrisson (40), 90% of patients acquired little or no periodontal breakdown during orthodontic treatment with fixed appliances, emphasizing the importance of repeated motivation and instructions of oral hygiene. The local changes in the oral ecosystem and the development of gingivitis during orthodontic treatment are reflected in changes in the composition of GCF (166, 167). The group of cytokines has attracted particular attention as their levels may increase rapidly with plaque accumulation and before the subsequent visible clinical inflammation, suggesting that they may have a potential role as early markers of gingival inflammatory changes (166). In the studies described in this Thesis on experimental tooth movement (chapters 6 and 7), no significant changes were observed on any of the clinical parameters during the whole experimental period. This is mainly due to the oral hygiene instructions given to each patient before and during the experimental treatment. In fact, meticulous plaque control and motivation for oral hygiene took place every week for all patients. This
allowed our research to focus on the mechanically induced inflammation within the bone and periodontal ligament. Although a weekly visit does not represent a common procedure, orthodontic patients should be aware of the importance of reaching high hygiene levels during orthodontic treatment. Furthermore, for the clinician it is important to identify patients at risk for periodontal disease and to control the periodontal status before any orthodontic appliance is placed.

Pain is a complex experience which accompanies orthodontic treatment (50). It is a subjective response showing large individual variations. The perception of orthodontic pain is part of the inflammatory reaction causing changes in blood flow following orthodontic force application. As a result of the inflammatory reaction, various biochemical mediators are released eliciting an hyperalgesic response. The painful response during the first days of orthodontic treatment has been related to the release of neuropeptides which in turn evoke the release of pro-inflammatory cytokines, such as IL-6, IL-8 and TNF-α mainly by dental pulp cells and by other cell lines (63, 72). The levels of such mediators return to normal values after 14 days. Orthodontic pain has a definite influence on compliance and daily activities of the patient. Many patients/parents consider initial lack of information about possible discomfort or pain, to be the major cause of poor compliance. Evaluation of pain is an important part of orthodontic treatment and the patients’ attitude towards orthodontics should be understood and discussed with the patient during the diagnostic phase. In our work described in chapter 3, a baseline interview was established in order to evaluate the patient’s previous experience of general and dental pain as well as the level of dental anxiety. Using the VAS, painful situations were proposed and the subjects had to place a mark on the line at a point that corresponds to the level of pain intensity. Regarding general pain, situations such as “cutting one’s finger”, “being stung by nettles”, “vaccination” were proposed. Regarding dental pain, situations such as “polishing”, “injection”, “drilling “, “scaling “ were proposed. Finally, dental anxiety was assessed based on 4 questions regarding 4 hypothetical dental situations (“how you feel the day before the dental appointment”, “how you feel 5 min before” etc). This questionnary is a very simple and rapid method to evaluate our patient’s perception towards pain. Furthermore, we showed that several biochemical markers detected in GCF, such as PGE$_2$, SP and IL-1β, were associated with pain intensity. The development of a chair-side biochemical test and a baseline interview
may help us to identify patients more “susceptible” to experience not just a discomfort but also high levels of pain during orthodontic treatment. This could further help us to manage pain and distress experienced by the patient. The administration of NSAIDs remains the preferred method for pain control (75). Low doses administered for one or two days in the initial stage of orthodontic treatment, or pre-operative analgesics administered at least one hour before every orthodontic procedure, will help the patient without affecting the tooth movement process.

Root resorption is a common sequela of orthodontic treatment (76-79). According to its severity, root resorption is classified to cemental or surface resorption, to dentinal resorption and to circumferential root resorption. The last, is clinically the most severe type, it is irreversible and starts at or near the apex and progresses coronally, resulting in root shortening. The most commonly resorbed teeth are the maxillary incisors. In most of the cases, resorption defects are confined to the apical third of the root because the apical third is covered with cellular cementum, which depends on active cells and has more supporting vasculature (207). This makes it more vulnerable to trauma and cell-injury related reactions. Furthermore, there is a decrease in the hardness of the cementum from the cervical to apical regions, making apical areas prone to resorption (208). About 5% of adults and 2% of adolescents are likely to have at least one tooth with resorption of more than 5mm during active treatment. Until now, several factors have been identified as predisposing factors for root resorption, such as age, gender, pre-existing root condition, type of tooth movement, amount and type of force as well as treatment duration (107-112).

An objective tool to identify teeth at risk of severe resorption is still missing. Until now, OPT and periapical radiographs are the only tools in the diagnosis of orthodontically induced root resorption. However, a certain degree of resorption is required before being detectable on the radiograph, thus making impossible its detection at an early stage (124, 125). In chapter 7, we showed that digitized periapical radiographs underestimated apical root resorption. Furthermore, in chapter 8, by using the CBCT as gold standard, we showed that OPT also underestimated orthodontically induced root resorption. We suggested that if signs of moderate root resorption are visible on OPT during the initial or middle phase of orthodontic treatment, the CBCT may be helpful in evaluating the severity of the situation and
help make the decision on continuation and possible modification of orthodontic treatment. Furthermore, in cases of impacted canines, CBCT may be particularly useful in revealing the presence and degree of root resorptions on the adjacent teeth and when orthodontic treatment combined with surgery is required. Otherwise, periapical radiographs should be obtained 6 months after the beginning of the orthodontic treatment in order to early detect the occurrence of apical root resorption.

Recently, the role of OPG and RANKL in osteoclast genesis and root resorption- both physiologic and pathologic- procedure has been documented (187, 188). Furthermore, these molecules have been identified in the gingival crevicular fluid mainly in relation to periodontal disease (189, 190). The development of a screen test based on the detection of OPG and RANKL in gingival crevicular fluid, before and during orthodontic treatment, may help to identify patients at risk for root resorption. In our study based on experimental tooth movement, buccal tipping forces were exerted, thus we postulate that the buccal cervical and lingual apical region have undergone tissue compression. The buccal apical and lingual cervical areas were under tension, and the mid-root regions had undergone a mixture of both compression and tension. Based on the hypothesis that the levels of OPG and RANKL may change in relation to the root resorption defects, the gingival crevicular fluid was collected in the compression areas during the whole experimental period. Identifying biological markers related to root resorption, may help to the development of a screen test before any changes on root length become obvious in the radiographs. Recently, research has been focused in identifying genes that control the cellular and ECM components associated with the events taking place during orthodontic tooth movement (115-118). A genetic test that will determine root resorption polymorphisms in patients prior to orthodontic treatment could contribute to the best clinical setting.

At present it is not possible for the clinician to predict patients at risk for severe root resorption during orthodontic treatment. Therefore, gingival crevicular fluid analysis before or during orthodontic tooth movement, may be useful for the prediction of individual’s response to orthodontic treatment and the related side effects such as root resorption and pain. In particular, the detection of patients at risk and the early detection of root resorption before the radiographic observation are important areas in orthodontics that need to be addressed in order to improve the orthodontic treatment.
13. REFERENCES


