Effects of orthodontic treatment on gingival crevicular fluid flow rate and composition: Clinical implications and applications

The gingival crevicular fluid (GCF) flow rate and composition vary according to the condition of the periodontal tissues. The levels of some of its constituents have been shown to correlate with the actual clinical measurements of periodontal disease progression and reflect changes occurring deep in the periodontium. During the course of orthodontic treatment, the forces exerted produce a distortion of the periodontal ligament extracellular matrix, resulting in alterations in cellular shape and cytoskeletal configuration. Such events lead to the synthesis and presence in the deeper periodontal tissues of extracellular matrix components, tissue-degrading enzymes, acids, and inflammatory mediators; induce cellular proliferation and differentiation; and promote wound healing and tissue remodeling. These changes may modify both the GCF flow rate and its components. Consequently, analysis of GCF samples may provide a better understanding of the biochemical processes associated with tooth movement and may help the clinician make therapeutic choices based on qualitative and quantitative information. (Int J Adult Orthod Orthognath Surg 2002;17:191–205)
chronic gingivitis. Moreover, a positive association has been reported between alkaline phosphatase and periodontal disease in humans, whereas in beagle dogs a positive association has been established between acid phosphatase and the level of inflammation. Consequently, GCF analysis may form the basis of a special test for the assessment of various clinical conditions, such as the active phases of destructive periodontal disease.

During orthodontic treatment, the forces exerted produce a distortion of the PDL extracellular matrix, resulting in alterations in cellular shape and cytoskeletal configuration and creating short-lived piezoelectric spikes that can lead to cellular activation by changing membrane polarity and ion channel activity. This distortion of the periodontal tissues also induces neuropeptide release from afferent nerve endings. Some of these molecules are vasoactive, causing vasodilation and migration of leukocytes into the extravascular space. These migratory cells synthesize and secrete a wide variety of cytokines and growth factors. In addition, as the capillaries are stretched or compressed excessively, tissue damage may occur. Such events and interactions lead to the synthesis and secretion of extracellular matrix components, tissue-degrading enzymes, acids, and local factors; induce cellular proliferation and differentiation; and promote wound healing and tissue remodeling. In vivo studies suggest that as biologic reactions progress at varying rates and intensities during different periods of treatment (earlier or later in active treatment, or earlier or later in retention), alternate combinations of biochemical molecules come into play. These combinations are dependent on alveolar remodeling dynamics, the cycles of injury and healing, and the composition of the PDL cell population at each period. Such changes in the deeper periodontal tissues may modify both the GCF flow rate and its components. Thus, analysis of GCF samples may help in assessing tissue status around teeth undergoing orthodontic movement and provide a useful instrument for the modification of orthodontic treatment procedures.

The present review was undertaken to investigate the effects of forces exerted on the PDL during orthodontic treatment on GCF flow rate and composition, and to consider any implications of such findings for clinical orthodontic procedures and decision-making.

**Methods for collecting GCF samples**

Several different methods have been used for the collection of GCF. For collection of predetermined volumes, microcapillary tubules are placed at the gingival crevice and either held at a particular site, or passed back and forth for 10- to 15-minute periods. This procedure can often be disruptive to the delicate crevicular epithelium, resulting in contamination of the native GCF with blood and serum. Collection of significant predetermined volumes can also cause an influx of serum from the gingival capillaries, leading to a dilution of the native GCF by serum, since the usual volume range in the undisturbed sulcus is between 0.5 and 1 µL. GCF can also be collected by placing a prewashed absorbent string into the gingival crevice. This method is also disruptive for the epithelium and can involve problems with the accurate weighing of small samples. Another common method is the placement of filter paper strips in the gingival crevice. Sample collection may last for a specific or an indeterminate period of time. Alternatively, the initial GCF sample can be discarded and the subsequent flow sample collected. Collecting the initial GCF is less disturbing to the crevicular epithelium and enables more rapid measurements of the GCF, thus decreasing the probability of altering the GCF by excessive contamination with serum. A paramagnetic bead method has been developed as an alternative procedure for such GCF components as tumor necrosis factor (TNF). This method does not require removal of any fluid from the gingival crevice and has been reported to give better recovery for TNF than paper strips or capillary tubules. The beads are covered with anti-TNF monoclonal antibodies and introduced into the gingival sulcus, where the antibodies form complexes with the TNF.
The beads are then retrieved with a special magnetic harvester. According to the researchers, this technique is advantageous because it allows for the capture of TNF directly from the crevice, eliminating the necessity of collecting a volume of crevicular fluid and then isolating and purifying the TNF from the fluid.36

Effects of orthodontic treatment on GCF flow rates

Two early studies reported contradictory results regarding GCF flow rates. Tersin38 found increased production of GCF during orthodontic treatment, in both a group receiving oral hygiene instruction and supervision, and a group not receiving such measures, while a later study39 found no significant difference between teeth undergoing orthodontic treatment and untreated contralateral teeth. Many studies have reported a significant correlation between plaque accumulation, gingival inflammation, and the volume of gingival exudate.1 However, in Tersin’s studies none of these factors were taken into consideration. This fact may account for the contradictory evidence, as the additional effect of orthodontic treatment itself on gingival fluid flow rate cannot be determined unless such influences are eliminated.

Later studies have taken gingival status into consideration. During orthodontic treatment, a significant increase in GCF flow rate has been found, unrelated to the presence of significantly more severe gingival inflammation.29,30,40 This increase was only partly attributed to increased gingival inflammation. Later, Pender and coworkers28 reported that the GCF tended to increase at both uninflamed and moderately inflamed sites, compared to similar sites before treatment. At early stages of retention, Last and coworkers30 demonstrated an increased GCF flow rate compared to an untreated control group. However, if samples are collected later in retention, as done by Pender and coworkers,28 GCF volumes at clinically healthy and mildly or moderately inflamed sites were much lower than at similar sites before treatment commenced. Consequently, these reports show that GCF collected by microcapillary tubules tends to increase in teeth undergoing orthodontic treatment. Later, as a new equilibrium situation in these tissues becomes established and consolidated during retention, GCF has been shown to decrease, becoming even less than from untreated teeth. The latter was attributed to inhibition by the orthodontic appliance of physiologic tooth micromovements existing in control teeth.

A recent study27 reports that the increase in GCF flow induced by orthodontic tooth movement begins earlier than the pronounced changes in GCF components. This finding suggests an immediate effect of orthodontic force on the blood vessels, rather than induction of biochemical changes in the extracellular matrix.

In contrast to the above, Uematsu and coworkers34 reported that the volume of fluid taken with filter paper strips from around the experimental tooth during orthodontic movement was nearly the same as from around healthy teeth. Corroborating evidence was produced by Miyajima and coworkers,41 who found no significant differences in GCF volume between treatment, retention, and control groups, although the mean value of the retention group was smaller than that of the others. None of the patients had signs of gingival inflammation.

Effects of orthodontic treatment on GCF composition

After exposure to orthodontic forces, tissue fluid movement is followed by strain to cells and the extracellular matrix and local damage to the PDL. Target cells in the PDL and the 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 reflected in the synthesis and secretion of extracellular matrix components and tissue-degrading enzymes and acids, resulting in modification of GCF composition.25
Mineralized tissue components and other markers of bone turnover

Glycosaminoglycans. GAGs form a group of minor GCF components. They are found in the extracellular matrix of mineralized and other connective tissues, and are negatively charged complex carbohydrates, linked covalently, in the native state, to a core protein to form proteoglycans. In the periodontal tissues, chondroitin-4-sulfate (CS) rather than the chondroitin-6-sulfate isomer predominates in gingiva, PDL, alveolar bone, and cementum. Dermatan sulfate and heparan sulfate (HS) are also present in the soft tissues but not consistently found in bone. The non-sulfated GAG, hyaluronic acid (HA), is distributed throughout the periodontal tissues and is present in particularly high amounts in gingiva. Changes in the GAG profile of GCF, especially in CS, have been associated with degrading changes in the deeper periodontal tissues.

Chondroitin sulfate. Last and coworkers carried out a cross-sectional quantitative investigation by cellulose acetate electrophoresis of samples collected simultaneously, with microcapillary pipette, from the mesial and distal surfaces of teeth in 3 groups of young persons (no treatment, active treatment with fixed appliances, or early in retention). Elevated CS levels were observed at the surface toward which movement was directed in teeth undergoing orthodontic treatment, compared with untreated controls. Later, in a longitudinal study conducted by Samuels and coworkers, GCF was collected with microcapillary tubules alternately from the mesial and distal surfaces of teeth in 2 groups of children, one undergoing therapy with fixed appliances and the other with functional appliances. Electrophoretic identification and densitometric separation of GAG components followed. Additionally, measurements of the vertical and horizontal components of movement observed were made on dental casts. An increase was reported in CS levels from teeth moved both vertically and horizontally that could not be attributed to the effects of plaque, gingival inflammation, or pocket probing depth, because their tendency to increase during treatment was not statistically significant. In addition, the movement of the canine into an extraction site did not have a significant effect on the GAG levels in GCF, and the researchers concluded that the vertical component of movement may be important in producing the change in CS levels in the GCF. Contrary to the findings of Last and coworkers, no significant difference in CS levels was found at the mesial and distal surfaces of teeth undergoing active movement, although at the distal surface CS levels tended to be higher. This finding was considered to be partially an effect of the alternate sampling from the tooth surfaces, unlike the simultaneous sampling by Last and coworkers. It is also likely that during the distal movement of the canine, a series of tipping and uprighting movements occurred, producing areas of resorption and deposition of bone on both sides of the canine root. A coexisting vertical component of movement induced bone deposition on both mesial and distal surfaces. As a result, absolute polarization of tissue activity to one side of the tooth was prevented. Using similar methods, Baldwin and coworkers produced findings corroborating the results of the first 2 studies and concluded that the increase in CS levels was related to the degree of movement and the presence of a vertical component. In contrast to the aforementioned studies, Pender and coworkers, using a research protocol similar to the previous studies, failed to observe a significant rise in CS levels during active canine retraction. This finding was possibly associated with the decrease noted in the vertical component of movement.

As to the origin of the CS component in GCF, the relative bulk and high concentration of CS in human alveolar bone suggests that this tissue may be the primary source of CS in GCF. Other studies report that the sulfated GAG content of alveolar bone is increased by orthodontic tooth movement and that bone resorption, stimulated in vitro by an extract of dental plaque, increases the release of sulfated GAG into the culture medium. These observations suggest that the elevated levels of GCF collected from teeth during active movement represent changes in the deeper rather than the superficial periodontal tissues.
Two of the previous studies evaluated CS levels during retention. At early stages of retention, Last and coworkers reported increased levels compared with untreated control teeth. Thus, it is possible that either those periodontal tissue changes that generally lead to increased GAG contents during active orthodontic treatment may persist in early retention, or periodontal tissues undergo further changes, reflected in the raised CS content. Such changes may possibly be induced by forces from occlusal contacts and surrounding muscles that produce further minor tooth movement. In addition, stretched collagenous fibers tend to reverse the original tooth movement.

In contrast, at later stages of retention, Pender and coworkers observed a decrease in CS content, possibly associated with the establishment of a new equilibrium status in deeper periodontal tissues. Moreover, after a prolonged period of retention, GAG metabolism and GCF production appear to be lower than in untreated teeth. It is possible that the inhibitory effects of the appliance on physiologic movements and accompanying periodontal tissue remodeling, by which a functional tooth position is attained and maintained, are represented in the changes in GAG composition of the GCF.

Hyaluronic acid. With regard to HA, Last and coworkers and Pender and coworkers found no significant increase in GCF around teeth undergoing active orthodontic treatment. However, Samuels and coworkers reported that the level of HA tended to increase in all fixed appliance groups—significantly so in those with a small amount of horizontal tooth movement only. No increase was reported for the functional appliance group, possibly associated with the minimal effect of the functional appliances on the relative position of the canines, in contrast to that of the fixed appliances.

Although it would seem likely that HA content is influenced by changes in gingival inflammation, as observed in vitro, only a weak association has been found. Therefore the possibility exists that particular tissue changes produced by the fixed appliance tooth movement leads to differential changes in the HA content within the GCF.

During early retention, Last and coworkers observed no significant increase in HA levels. At later stages, a decrease was even reported.

Heparan sulfate–like component. Last and coworkers noted an HS-like component in the GCF in the retention group, whose origin and significance is uncertain. HS may influence several phenomena, such as cell adhesion and cell growth.

Other mineralized tissue components. Other mineralized tissue components are used routinely to provide information on bone resorption and formation in the evaluation of bone disorders such as Paget’s disease, hyperparathyroidism, and osteoporosis. Such markers of bone turnover were examined in relation to orthodontic tooth movement.

Osteocalcin. Osteocalcin is a non-collagenous matrix protein of calcifying and calcified tissue. It is produced by osteoblasts and has been described as the most specific marker of osteoblast function. Structurally, it binds to both major bone components (collagen and apatite) and is believed to play a role in both bone resorption and mineralization. Osteocalcin has been found in GCF from patients with periodontal disease, and increases in GCF osteocalcin concentration were associated with high bone turnover, assessed by digitized radiography and bone-seeking radiopharmaceutical uptake. On the other hand, Lee and coworkers found no statistically significant differences between osteocalcin levels in diseased and healthy sites in adult subjects. Recently, it has been proposed that osteocalcin has an additive effect on the rate of orthodontic tooth movement through the enhancement of osteoclastogenesis on the pressured side.

Griffiths and coworkers examined osteocalcin levels during orthodontic tooth movement. They collected GCF samples from the distal surfaces of maxillary canines from 20 patients with fixed appliances during various stages of treatment. A filter paper strip was inserted in the sulcus for 5 s, and after 1 minute another strip was placed for another 5 s. After a 30-s interval, a final strip was inserted for 30 s. Osteocalcin was detected during every stage of treatment. Concentrations and quantities
measured exhibited a wide variation between subjects. No pattern in osteocalcin fluctuations was observed. According to researchers, osteocalcin may be a constituent of GCF associated with the developing dentition. Therefore, it remains to be established whether osteocalcin GCF levels can be used as markers of bone turnover.

Pyridinium crosslinks of bone collagen. The pyridinium derivatives, pyridoline (Pyr) and deoxypyridoline (dPyr), are structural elements that bind together collagen chains (referred to as crosslinks). Pyr is abundant in skeletal tissues, whereas dPyr is a minor component found predominantly in bone and dentin. These 2 molecules are used as markers to evaluate bone resorption in such cases as Paget’s disease and primary hyperparathyroidism. Meng and coworkers detected the pyridinium crosslinks in GCF from tooth sides described as “disease active.” Recently, it has been suggested that concentrations of pyridinium derivatives in urine may be an alternative method of estimating mandibular growth.

Griffiths et al. failed to detect pyridinium crosslinks during orthodontic tooth movement. This failure was attributed to either lack of Pyr and dPyr production associated with orthodontically induced bone remodeling or to erroneous GCF sampling timing when maximum bone remodeling had already occurred. Another factor was considered to be the low sensitivity of the assay system due to the small volumes available.

Other markers of bone turnover. Apart from the aforementioned, other markers of bone turnover have been studied in relation to orthodontic movement.

Lactic and citric acid. It has been suggested that, during orthodontically induced bone resorption, the demineralization process is mediated by both lactic and citric acid. Their production by osteoclasts is believed to bring about the pH changes necessary for apatite crystal removal.

Miyajima and coworkers collected GCF samples from the 6 maxillary anterior teeth of 34 individuals (undergoing treatment, retention group, and control group) whose periodontal condition was judged to be fairly healthy. The samples were collected with filter paper strips (3 for each tooth) inserted for 10 minutes into the labial gingival sulcus. Data analysis showed that both lactic and citric acid content was elevated in the treatment group, and the researchers concluded that these acids can be good parameters in monitoring tooth movement intensity as well as stability at the retention phase.

Mediators of the inflammatory processes

Storey proposed that the early phase of tooth movement involves an acute inflammatory response characterized by periodontal vasodilation and migration of leukocytes out of the capillaries. Recent research has led to the hypothesis that after mechanical stimulus, inflammatory mediators are released, triggering the biologic processes associated with alveolar bone resorption and apposition. Among the local biochemical mediators are the cytokines, which are secreted by mononuclear cells and leukocytes. Cytokines can provoke the synthesis and secretion of numerous substances that form the molecular basis for cell-to-cell communication, including prostaglandins and growth factors, thus interacting directly or indirectly with bone cells.

Prostaglandins, other arachidonic acid metabolites, and interleukins. Prostaglandin E (PGE) has long been considered a major stimulator of bone resorption. In the very early stages of treatment, PGE₂ may be a pivotal molecule, acting as both autocrine and paracrine. Within a short time, as periodontal tissues become progressively more strained by the applied forces, cells are subjected to other first messengers of inflammation, products of cells of the nervous and immune systems. These signal molecules lead to a cytoplasmic increase of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). PGE₂ remains an important cause of these changes. However, many neurotransmitters, cytokines, and growth factors also utilize a cyclic nucleotide route. Therefore it is difficult to attribute such changes to any specific biochemical factor. Rather, they are subjected to the stimulatory effect of a number of available signals. Corroborating
evidence has been produced by Sandy and Harris, who observed a significant decrease only in osteoclast numbers, suggesting that prostaglandins do not act alone, although inhibition of tooth movement by indomethacin has been reported. Other arachidonic acid metabolites, such as leukotrienes, may account for this discrepancy as well. Experimental evidence supporting this contention was presented by Mohammed and coworkers; inhibition of leukotriene synthesis can significantly reduce orthodontic tooth movement.

PGE$_2$ production is partly modulated by IL-1. There exist 2 forms of IL-1, alpha and beta, coded by different genes but having similar activities, such as chemotactic properties, regulation of cell metabolism of connective tissue, stimulation of prostaglandin release from fibroblasts, and enhancement of bone resorption. These activities seem to be directly relevant to periodontal destruction. The 2 forms of IL-1 are produced mainly by monocytes and macrophages. IL-1$\alpha$ is primarily cell-associated and is not commonly found in serum or body fluids. IL-1$\beta$ is mostly involved in bone metabolism and can be identified in human GCF. Saito and coworkers showed that PDL cells respond to mechanical stress (in vivo and in vitro) by increased production of PGE, a response enhanced by IL-1$\beta$. Thus, after the application of mechanical forces, cells in the PDL may produce sufficient amounts of PGE and IL-1$\beta$ to diffuse into the GCF.

Apart from the IL-1 receptor agonists, a receptor antagonist has been described and isolated (IL-1ra), which blocks IL-1 activity in vivo and in vitro. Experimental findings suggest that IL-1ra may play an important role in alveolar bone resorption in periodontitis. To explore the correlation between IL-1 agonists and antagonist receptors, the IL-1 activity index (IL-1AI) was introduced.

Another cytokine of the interleukin family with a stimulatory effect on bone remodeling and osteoclast formation is IL-6, a protein involved in the acute phase of the inflammatory process. IL-6 is produced by fibroblasts in the human PDL and osteoblasts. The latter are not affected functionally (in terms of proliferation, alkaline phosphatase activity, osteocalcin production, and PGE$_2$ release), suggesting that IL-6 acts as a paracrine during orthodontic tooth movement, acting on osteoclast formation.

Tzannetou and coworkers collected GCF samples from teeth undergoing rapid palatal expansion using filter paper strips. Samples from the mesiopalatal aspects were analyzed for total IL-1$\beta$ using an enzyme-linked immunosorbent assay (ELISA) method. The researchers observed an early increase in IL-1$\beta$ levels in response to orthodontic/orthopedic forces. These changes were attributed to mechanical stimulation and to bacterially induced inflammation, as the Plaque Index remained low throughout the study. In the post-activation period, increased levels of IL-1$\beta$ were observed as well. This finding may be attributed either to the relapse tendency of the 2 maxillary segments and the bone remodeling around the anchor teeth, or to alterations in palatal and buccal soft tissues.

An earlier study also reported increased levels of PGE and IL-1$\beta$ adjacent to teeth undergoing orthodontic tooth movement with fixed appliances that were not correlated with bacterially induced inflammation. Samples were collected using paper strips. On activation, IL-1$\beta$ levels increased rapidly. The PGE production peaked later, suggesting a stimulatory effect of IL-1$\beta$ on PGE. This finding supports those by Saito and coworkers. The decrease noted later could be attributed to feedback inhibition by increased levels of PGE.

Corroborating results were reported by Uematsu and coworkers. They took samples from 12 subjects undergoing orthodontic treatment. For each subject, a canine undergoing distal movement was used as the experimental tooth, and the contralateral and the antagonistic canines served as the controls. Samples were collected from the distal surface of each tooth. Paper strips were placed for 30 s. Then, after a 1-minute interval, a second strip was placed at the same site. IL-1$\beta$ and IL-6 concentrations were significantly higher in the experimental group than in the controls at 24 hours after initiation of the experiment.

The same finding of a rise in IL-1$\beta$ was reported by Iwasaki and coworkers. GCF
samples were obtained with filter paper strips from the mesial and the distal surfaces of the canine from 7 patients undergoing orthodontic treatment involving maxillary first premolar extractions and distal bodily movement of the maxillary canines. Paper strips were inserted for 30 s then removed. After 1 minute, a second strip was placed for another 30 s. During the experimental period, the direction and rapidity of tooth movement were also measured. IL-1β concentrations spiked within 3 days after loading and then dropped to baseline levels by 14 to 28 days. The IL-1β concentrations showed a periodicity of approximately 28 days. IL-1ra also peaked after 3 days. However, no periodicity was evident. When the correlation was measured between a modified IL-1Al, defined by the equation AI = Experimental (IL-1β/IL-1ra)/Control (IL-1β/IL-1ra), and tooth velocity, the results showed that the velocity of tooth movement in an individual was related to their AI. Since the correlation was not so strong, researchers proposed further modification to the AI that would reflect the effects of concentrations of other factors involved in bone remodeling, rather than just IL-1β and IL-1ra.

During the earlier stages of tooth movement, IL-1β may derive from many periodontal cell types. Thus, the increase in IL-1β increase may be associated with undermining resorption following hyalinization induced by heavy forces. IL-1β is thought to be secreted primarily by macrophages, which are involved in the phagocytosis of necrotic tissue and degranulation of hyalinized bone and contribute to the repair process. However, macrophage accumulation in compressed areas has been observed in later stages after initiation of tooth movement. It may be possible that IL-1β is released from cell types other than macrophages, such as the osteoblasts, as an immediate response to mechanical stress. IL-1β produced by the osteoblasts leads to production of cAMP, stimulation of PGE production, and triggering of the bone resorption process by the osteoclasts. Alternatively, IL-1β can act as a chemotactic factor for the polymorphonuclear neutrophils, associated with the increase in β-glucuronidase observed later in treatment. At later stages of orthodontic movement, only osteoclasts and adjacent mononuclear cells in resorption sites stained distinctly for IL-1β. Perhaps at this stage it is not possible to detect more subtle alterations in mediator levels that are occurring primarily in the bone.

Transforming growth factor-β1. Transforming growth factor-β1 (TGF-β1) is a family of polypeptides involved in cellular proliferation, differentiation, and migration. The most abundant isoform in mammalian species is TGF-β1. Several kinds of cells are known to produce it. Among these, it is speculated that TGF-β1 is produced by cells within the periodontium, especially osteoblasts and fibroblasts. TGF-β1 has been shown to stimulate matrix protein synthesis, to be implicated in the rapid remodeling of periodontal tissues, and to have multiple effects on bone cells depending on their phenotype and stage of differentiation. Therefore, it may act as a factor linking bone resorption to bone formation. In contrast, TGF-β1 is also a potent immunomodulatory molecule, suppressing the proliferation and differentiation of most cells of B- and T-cell lineages in vitro, antagonizing the effects of inflammatory cytokines such as IL-1β or TNF-α, and suppressing expression of receptors for IL-1β. Therefore it may provide a negative feedback circuit that inhibits activation, proliferation, and various functions of immune cells within the PDL.

Uematsu and coworkers reported the presence of TGF-β1 in GCF during orthodontic tooth movement. Samples were collected from the distal surface of canines with healthy periodontium using filter paper strips, and TGF-β1 was then estimated by the ELISA method. The researchers had previously ensured that their method did not allow contamination of GCF collected with plasma or salivary proteins. TGF-β1 showed a rapid and transient increase associated with cytokine induction during the early stages of tooth mobilization. This observation is unlikely to have been a result of local gingivitis, as there is no tendency for gingivitis to increase during orthodontic tooth movement. Moreover, it could not be the result of an increase in GCF flow rate, as the volume collected in
the predetermined time from the experimental tooth was nearly the same as that from around a healthy tooth. As TGF-β1 is involved in regulation of several factors during bone resorption and apposition, the elevation of these cytokine levels may reflect an early stage of the biologic responses induced by mechanical stress.

Tumor necrosis factor-α. TNF-α has been shown to be an early modulator of bone resorption.59,76 This molecule is synthesized mainly by monocytes and macrophages, and it is a major participant in inflammation.25 TNF produced from monocytes and macrophages induces differentiation that leads to a greater number of osteoclasts. Alternatively, resting osteoclasts are activated by the induction of IL-1β production by the fibroblasts.77 TNF also induces fibroblasts to produce PGE₂ and procollagenase.25 In general, TNF promotes osteoclastic activity and inhibits osteoblastic stimulation of bone formation.78

Lowney and coworkers36 measured TNF-α before and after the application of an orthodontic force, assuming that TNF-α generated at the site of bone turnover in the human PDL can be expelled toward the gingival crevice in response to orthodontic force systems. Therefore, orthodontic forces were used in 2 steps. Initially, forces were applied to induce bone turnover for at least 1 month. Later, additional forces (T-spring or elastic ligature) were applied to compress the PDL to promote the cervical movement of the biochemical components formed. To recover TNF-α paramagnetic beads coated with monoclonal antibodies were introduced into the gingival sulcus. The samples were collected from 50 teeth undergoing orthodontic treatment in 20 patients. The procedure took place before and 5 minutes after activation, and since TNF levels are variable from site to site and subject to subject,79 each site served as its own control before force was applied to the tooth. After quantification using an immunochemical assay, an increase in the mean level of TNF was reported; this corroborated the theory that it plays a role in tooth movement in human beings. Local subclinical gingivitis could be another source of this TNF. However, this is unlikely, since the same sample site provided the control and experimental data after only 5 minutes. The differences reported between the 2 groups of results may arise from the difference between the types of mechanics used.

Epidermal growth factor. Epidermal growth factor (EGF) is another cytokine possibly associated with bone remodeling. It is produced by fibroblasts and stromal cells. The latter have been reported to be precursors for osteoclasts.71 Uematsu and coworkers,72 in 12 patients, reported a transient elevation of EGF levels at 24 hours after the application of mechanical stress in the PDL of the experimental tooth.

β₂-microglobulin. β₂-microglobulin (β₂-MG) is considered a mediator with a considerable role in the inflammatory response, because of its association with major histocompatibility complex, class I, as well as because of its similarity in amino acid sequence to the constant region of the immunoglobulin chain. Although it is produced in other tissues besides bone, β₂-MG enhances the biologic action of insulin-like growth factor-I (IGF-I).71 IGFs are a family of peptides that promote cell proliferation and differentiation and have insulin-like metabolic effects. They have been associated with stimulation of the osteoblastic function.25 Therefore, an increase in β₂-MG concentration leads to enhancement of bone deposition activity. Uematsu and coworkers71 found an increase in β₂-MG levels in the GCF after mechanical orthodontic stimulation.

Tissue-degrading enzymes

Based on the cycles of injury and healing, several combinations of tissue-degrading or tissue-repairing enzymes come into play, following the mechanical perturbation induced by orthodontic forces.

Interstitial collagenases. The degradation procedures in periodontal tissues during orthodontic tooth movement have been suggested to initiate by the action of interstitial collagenases (ICs).80 Two types of ICs exist, the fibroblast type and the neutrophile, secreted as latent procollagenases respectively by periodontal fibroblasts and infiltrating neutrophils.81 These 2 forms are structurally homologous and exhibit similar catalytic and antigenic properties. However, characteristics
such as relative susceptibility to non-proteolytic and proteolytic activators, relative susceptibility to tetracycline inhibition, and relative ability to degrade interstitial collagen can be useful in differentiating between them. Sorsa and coworkers investigated the IC activity in GCF during orthodontic tooth movement. Their investigation involved 6 patients under orthodontic treatment for 5 to 7 months. They took samples from 11 canines retracted with a PG-retractor spring. Incisors of the same patients served as controls. Samples were collected from apposition and resorption sides. No signs of gingival bleeding, periodontal inflammation, or pocket formation were observed. Total collagenase activity in the test group was about 10 times higher than in control samples. As tested by specific doxycycline inhibition, neutrophile collagenase was predominant in resorption-side samples. The apposition-side samples contained slightly more fibroblast-type collagenase. The results are indicative of the collagen-degrading procedures in the periodontal tissues as a result of orthodontic forces.

β-glucuronidase. The lysosomal enzyme β-glucuronidase is a marker of primary granule release from polymorphonuclear leukocytes. It contributes to the hydrolysis of glucuronic acid, which forms part of HA, and together with hyaluronidase takes part in the degradation processes of mucopolysaccharides of connective tissues.

Tzannetou and coworkers, using filter paper strips, collected GCF samples from teeth undergoing rapid palatal expansion with a Hyrax appliance. Samples from the mesiobuccal aspects were analyzed for total β-glucuronidase using time-dependent fluorometry. A significant β-glucuronidase increase was observed 2 weeks after activation of the appliance, in contrast to the pattern of IL-1β increase. This finding could not be a result of plaque accumulation, as plaque was maintained at a low level. The absence of an early response by polymorphonuclear leukocytes, in contrast to what is observed in bacteria-induced inflammation, may be attributed to lack of bacterial chemotactic factors. IL-1β has been found to act as a chemotacting factor for the polymorphonuclear cells. Thus the early IL-1β rise reported in the same study may be responsible for the elevated levels at later stages of treatment. However, Lilja and coworkers did not observe any increase in polymorphonuclear cell influx at the compressed PDL during a 4-week period of tooth movement.

Acid and alkaline phosphatase. Monitoring acid and alkaline phosphatase activity in tissues and serum is a common means of assessing bone turnover in human subjects. Bone resorption induces elevations in acid phosphatase activity, whereas bone formation is associated with higher alkaline phosphatase activity. Histochemical studies have suggested that alkaline phosphatase may be important in monitoring orthodontic tooth movement. Others have reported increased acid and decreased alkaline phosphatase at pressure sites; with tension, both acid and alkaline phosphatase activity increase. In serum, phosphatase activity correlates with bone turnover during tooth movement and is time dependent. Acid phosphatase presents an early increase, followed by reversal and a later peak in alkaline phosphatase. As an exudate, GCF reflects metabolic changes in the periodontal tissues; therefore, it is likely that phosphatase activity in GCF can be influenced by alterations in alveolar bone turnover.

Insoft and coworkers performed a longitudinal study on 3 subjects, collecting samples with filter paper strips from 1 maxillary first premolar during buccal tipping. Data showed early elevations in alkaline phosphatase activity during the time when little tooth movement occurs and an increase in acid phosphatase activity that coincided with maximum tooth movement. The same researchers studied phosphatase activity cross-sectionally. Samples were taken from left and right maxillary first molars, from both mesial and distal surfaces. A primary observation was that alkaline phosphatase activity may, in part, be affected by inflammation. Data analysis in relation to total appliance duration revealed an apparently oscillating pattern in each enzyme’s activity, suggesting the alveolar bone remodeling dynamics, characterized by periods of activation, resorption, reversal, and formation. First, a peak of
Acid phosphatase was observed, followed by a peak in alkaline phosphatase activity. This pattern of oscillating changes continued as treatment time increased. Similar bone remodeling kinetics have been reported in animal models. It is possible that orthodontic appliance activation may result in synchronization of multiple bone remodeling cycles, thus enabling the distinguishing of their various stages by the study of biochemical changes in the GCF. It is important to stress that the previous data were cross-sectional and the pattern created was only apparently longitudinal. It remains for a true longitudinal study to corroborate the above report. Another observation was that no phosphate changes were discernible in the GCF for a certain period of time. This finding may suggest either an insufficient amount of bone remodeling before that period, or a lag period between mechanical loading and the attainment of maximal response.

Clinical implications and applications

Research regarding the mechanism and evaluation of bone metabolism in orthodontic tooth movement is related to its potential pharmacologic modulation. Anti-inflammatory, analgesic, and antipyretic drugs, when prescribed to control the pain and discomfort associated with orthodontic appliance activation, could decrease the rate of tooth movement. These drugs inhibit cyclooxygenase activity and therefore the synthesis of prostaglandins. Studies of animals treated with prostaglandin inhibitors reported decreased osteoclastic activity. When lipoxygenase inhibitors were used to block leukotriene production, a significant decrease in the rate of tooth movement was observed. In these studies, higher than therapeutic doses were used for periods of days. However, in clinical practice, these drugs are used at lower doses for only 1 or 2 days after activation. From another perspective, local administration of PGE or IL-1β could augment the rate of tooth movement. In fact, this has been observed in both monkeys and human beings after local administration of PGE, with an absence of macroscopic, radiographic, or local side effects. Also, local or systemic deleterious effects have not been observed after local administration of IL-1β.

Furthermore, when an association between clinical measures and measures of tissue remodeling in the GCF has been established, the latter could be clinically useful to biologically monitor and predict the outcome of orthodontic treatment. Thus, appliance management could be based on individual tissue response, and the effectiveness of treatment could be improved by determining the optimum force magnitude that would provide most rapid tooth movement with the fewest side effects (bone loss, gingival recession, and root resorption). GCF analysis may also provide a means of assessing patient compliance.

In addition, the longitudinal effects of different force delivery systems on tooth movement can be investigated by evaluating changes in the GCF. By such means, it has been suggested that in vivo, nickel-titanium archwires cause relatively constant periodontal tissue perturbation, as would be expected of archwires with super-elastic properties. Despite contrasting evidence obtained in vitro.

Alternatively, GCF analysis may provide a noninvasive method allowing modification of retention procedures after orthodontic treatment. The nature and extent of post-treatment relapse following retention has been found to be unpredictable. Since orthodontic tooth movement brings about changes in GCF composition, the absence of such changes may show that bony changes have been curtailed. Therefore, the point where the chance of relapse is minimal can be determined.

However, reservations exist on whether direct extrapolations can be made from the results mentioned to everyday clinical practice. To evaluate the effects of mechanical orthodontic stimuli on GCF flow and composition, research protocols took into consideration plaque accumulation and gingival condition. Thus, the effect of bacteria-induced inflammation could be diminished. Nevertheless, in clinical practice, such an effect cannot be controlled, as it depends on patient compliance and dental hygiene, as well as mechanical irritation from the band or the cement.
it is generally accepted that gingival health is compromised, especially when orthodontic bands are worn. Many studies have reported a significant correlation between plaque accumulation/gingival inflammation and the volume of gingival exudate. In addition, some GCF components, such as alkaline phosphatase, are influenced in part by gingival inflammation. Others, such as CS, are not detected at sites of chronic periodontitis, although they are present at sites of advanced periodontal disease involving alveolar bone and PDL. Consequently, not only should the most appropriate biochemical molecular indicator be measured, but also the great importance of maintaining a high level of oral hygiene must be emphasized. Otherwise, biochemical molecule quantities and GCF volumes may be influenced.

Another limitation of GCF-based studies arises from the inherent variability of the quantities of the components and GCF volume, which is partly attributable to the method of collection used. The standard practice for each tooth under control is gentle washing with water or supragingival plaque removal, isolation with cotton rolls, and gentle drying with an air syringe. This procedure by itself may disturb the tissues and capillary permeability (although it is unlikely), resulting in serum influx and influencing GCF volume and components, especially when gingival status is compromised. In addition, GCF sampling with microcapillary tubules often disrupts the crevicular epithelium and results in contamination of the native GCF with blood and serum. In addition, collection of standardized volumes can cause a disruption, leading to a serum influx into the crevice and a subsequent dilution of the native GCF by serum. The use of filter paper strips for the collection of native GCF is less disturbing to the crevicular epithelium and enables more rapid measurements of GCF. Both of these advantages also further reduce the probability of altering the GCF by excessive contamination with serum. The volume of GCF can be accurately determined between 0.2 and 0.4 pL with the Periotron. However, the accuracy of this instrument below 0.1 pL is questionable. As a result, small errors in volume determination can lead to large errors in estimating the final concentrations when the total volumes collected are small. According to Lamster and coworkers and Insoft and coworkers, measurement of the total quantity of mediator collected for a standardized short period of time will allow for more sensitive detection of site-to-site and patient-to-patient GCF differences without significant contamination with serum. It would also be useful to ensure that crevicular fluid proteins were not contaminated with plasma or salivary proteins, indicating that the collection method used was suitable, as carried out by Uematsu and coworkers.

Another factor influencing the choice of method used is the particular component studied. It has been reported for TNF that only 50% can be recovered when using filter paper strips. This finding led to the evolution of a new method of collection. In general, since many procedures used were based on those used for studying other biologic fluids, such assays must be evaluated for their sensitivity. Not all standard assays have the required sensitivity and specificity for GCF analysis, but the overall predictability can often be improved without changing the basic assay structure.

Conclusions

Studies of the effect of orthodontic forces on GCF flow rates and composition have produced promising results. However, further studies are needed to establish procedures useful for clinical monitoring of biologic processes in the deeper periodontal tissues and the GCF during orthodontic tooth movement in human beings. More sensitive and more accurate methods of detection need to be explored. Studies should include longitudinal sampling of a large number of experimental teeth at frequent intervals to qualify and quantify the effects of orthodontic forces on the periodontal tissues, early and late, during the whole course of orthodontic treatment. In addition, GCF changes should be correlated with the amount and type of movement, as this factor has been stressed by some studies. More particularly, the effect of the vertical component of movement needs to be
References


