**Orthodontics**

**Review Article**

**SIGNALING MOLECULES AND GENETIC MECHANISMS INVOLVED IN ORTHODONTIC TOOTH MOVEMENT**

1 NABEEL SHDEIFAT, BDS (Jordan), JDB (Jordan)
2 IBRAHIM SULEIMAN AL-SHORMAN, BDS, JDB

**ABSTRACT**

The objective of this review is to highlight recent developments in cellular, molecular, tissue, and genetic reactions in response to orthodontic force application. Histologic, histochemical, and immunohistochemical studies in the 20th century and the early 21st century demonstrated that many phenomena, both physical and biologic, are involved in tooth movement. When mechanical forces are applied, cells, as well as the extracellular matrix (ECM) of the (periodontal ligament) PDL and alveolar bone, respond concomitantly, resulting in tissue remodeling. During the early phases of orthodontic tooth movement, PDL fluids are shifted, and cells and ECM are strained. In areas where tension or compression evolves under the influence of the orthodontic appliance, vasoactive neurotransmitters are released from distorted nerve terminals. In the PDL, most terminals are near blood-vessel walls. Therefore, the released neurotransmitters interact first with capillary endothelial cells. In response, the endothelial cells express receptors that bind circulating leukocytes, promoting their migration by diapedesis out of the capillaries. These migratory cells secrete many signal molecules, including cytokines and growth factors, some of which might be categorized as inflammatory mediators, that stimulate PDL and alveolar bone lining cells to remodel their ECM. This force-induced remodeling facilitates movement of teeth to areas in which bone had been resorbed.

Key Words: Force-induced remodeling, cytokines, growth factors, tooth movement.

**CYTOKINES IN ORTHODONTIC TOOTH MOVEMENT**

Cytokines are extracellular signaling proteins that act on nearby target cells in low concentrations in an autocrine or paracrine fashion in cell-to-cell communications. Cytokines that were found to affect bone metabolism, and thereby orthodontic tooth movement, include interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNFα), gamma interferon (IFNγ), and osteoclast differentiation factor (ODF). The most potent among these is IL-1, which directly stimulates osteoclast function through IL-1 type 1 receptor, expressed by osteoclasts. Secretion of IL-1 is triggered by various stimuli, including neurotransmitters, bacterial products, other cytokines, and mechanical forces.1 IL-1 has 2 forms—α and β—that code different genes. These interleukins have been reported to have similar biologic actions, systemically and locally. These actions include attracting leukocytes and stimulating fibroblasts, endothelial cells, osteoclasts, and osteoblasts to promote bone resorption and inhibit bone formation.2 Osteoblasts are target cells for IL-1, which in turn conveys messages to osteoclasts to resorb bone.1 Tuncer et al3 reported increased levels of IL-8 at PDL tension sites and proposed it to be a triggering factor for bone remodeling. TNFα, another pro-inflammatory cytokine, was shown to elicit acute or chronic inflammation and stimulate bone resorption. Recent studies4,5,6,7 have shown that TNFα directly stimulates the differentiation of osteoclast progenitors to osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF). Davidovitch et al4 and Saito et al5 demonstrated marked increases in the staining intensity for IL-1 and TNFα in cells of the PDL and alveolar bone during orthodontic tooth movement in cats.

Recent research by Alhashimi et al6,7 focused on the role of IFNγ during bone remodeling as part of orthodontic tooth movement. IFNγ is better known as a potent inducer of major histocompatibility complex antigens in macrophages, which is an early marker of immune activation during inflammation. It also evokes the synthesis of other cytokines, such as IL-1 and TNFα. These cytokines were shown to induce production of

1 Specialist Orthodontics, King Hussein Medical Center, Amman–Jordan
2 Specialist Orthodontics, King Hussein Medical Center, Amman–Jordan. Correspondence address: Dr N Al-Shdyfat, (KHMC), Tel: 0777742722, E-mail: nshdyfat@yahoo.com

Received for Publication: August 18, 2014
Revision Received: August 30, 2014
Revision Accepted: September 02, 2014
nitric oxide, a potentially important osteoblast-osteoclast coupling factor. Alhashimi et al.\(^1\) reported that, during orthodontic treatment, IFN\(\alpha\) can cause bone resorption by apoptosis of effector T-cells.

The role of cytokines of the RANKL/RANK/OPG system in inducing bone remodeling was demonstrated recently.\(^8\) The TNF-related ligand RANKL (receptor activator of nuclear factor-Kappa ligand) and its 2 receptors, RANK and osteoprotegrin (OPG), have been shown to be involved in this remodeling process. RANKL is a downstream regulator of osteoclast formation and activation, through which many hormones and cytokines produce their osteo-resorptive effect. In the bone system, RANKL is expressed on osteoblast cell lineage and exerts its effect by binding the RANK receptor on osteoclast lineage cells. This binding leads to rapid differentiation of hematopoietic osteoclast precursors to mature osteoclasts. OPG is a decoy receptor produced by osteoblastic cells, which compete with RANK for RANKL binding. The biologic effects of OPG on bone cells include inhibition of terminal stages of osteoclast differentiation, suppression of activation of matrix osteoclasts, and induction of apoptosis. Thus, bone remodeling is controlled by a balance between RANK-RANKL binding and OPG production. It has been suggested that OPG exists in both membrane-bound and soluble forms, and that its expression is up-regulated by CD40 stimulation. CD40 is a cell surface receptor that belongs to the tumor necrosis factor (TNF) receptor family.\(^9\) It can be seen in a variety of cells, such as B-lymphocytes, monocytes, dendrite cells, IL-6- and IL-8-secreting cells, such as endothelial cells, basophils, epithelial cells, and fibroblasts. It was found recently that CD40-CD40L (cellular responses mediated by CD40 are triggered by its counter receptor CD40L, which also belongs to the TNF gene family) interaction appears to be an active process during orthodontic tooth movement and that orthodontic force induces T-cell activation.\(^10\) Such activation might be involved in the induction of inflammatory mediators and subsequent bone remodeling. Kanzaki et al.\(^11\) reported recently that OPG gene transfer to periodontal tissues inhibited RANKL-mediated osteoclastogenesis and inhibited experimental tooth movement in rats. The number of reports cited above makes it clear that bone remodeling, particularly bone resorption, is regulated by cytokines released in response to the orthodontic force.

**GROWTH FACTORS**

Bone contains abundant amounts of transforming growth factor \(\beta\) (TGF\(\beta\)), which includes TGF\(\beta\)1, activins, inhibins, and bone morphogenetic protein.\(^12\) This small polypeptide is produced by several cell types, such as fibroblasts and osteoblasts, and is deposited in the ECM in a latent form. The richest sources of TGF\(\beta\) are platelets and bone, and it attracts monocytes and fibroblasts, and stimulates angiogenesis in vitro.\(^13\) These factors are involved in many biologic activities, including cell growth, differentiation, and apoptosis, as well as in developmental processes and bone remodeling.\(^1\) TGF\(\beta\) has been shown to enhance osteoclast differentiation in haemopoietic cells stimulated with RANKL and M-CSF.\(^14\) More recently, a family of signal transducer proteins has been identified, presenting a mechanism through which TGF\(\beta\) (specifically bone morphogenetic proteins) can signal from the cell membrane to the nucleus.\(^15\) The signal transducer protein families are phosphorylated by cell surface receptors with serine/threonine kinase activity and in this state translocate to the nucleus. Then, inside the nucleus, the transcription factors produce cellular responses to TGF\(\beta\).\(^16\) Isoforms of TGF\(\beta\) (TGF\(\beta\)1, \(\beta\)2, and \(\beta\)3), which are in latent form, are abundant in bone matrix. ten Dijke et al.\(^17\) demonstrated increased DNA synthesis by these 3 isoforms in osteoblast-rich cultures in fetal rats. These isoforms are also shown to enhance synthesis of collagen and noncollagenous proteins. Davidovitch et al.\(^1\) demonstrated TGF\(\beta\) immuno-reactivity in cat PDL cells and alveolar bone osteoblasts during orthodontic tooth movement. They reported that enhancement can occur as early as 1 hour after force application. The unstressed PDL and alveolar bone show negative or no staining for TGF\(\beta\).

The functions of 2 other growth factors—fibroblast growth factor (FGF) and insulin-like growth factor (IGF) — are similar.\(^1\) The target cells of FGFs include fibroblasts, endothelial cells, myoblasts, chondrocytes, and osteoblasts. Two forms of FGF were demonstrated: \(\alpha\)FGF (acidic PI) and \(\beta\)FGF (basic PI). Since \(\beta\)FGF and \(\beta\)FGF lack a signal sequence, they are sequestered in the cells responsible for their synthesis and are released only when there is a disruption of the plasma membrane. A recent report discussed plasma membrane disruption in PDL tension sites after orthodontic force application. This effect, demonstrated with the help of albumin uptake by PDL cells, suggests that plasma membrane disruption could promote uptake and release of large signaling molecules.\(^18\) Globus et al.\(^19\) reported that bone cells can synthesize \(\beta\)FGF and secrete it into the surrounding ECM, where it might act as an autocrine or a paracrine signal. Noff et al.\(^20\) demonstrated increased DNA synthesis, alkaline phosphatase activity, and formation of bone-like nodules, when rat bone marrow cells were treated with \(\beta\)FGF in vitro.

IGF I and II might also be of relevance during tooth movement. This family of polypeptides promotes cell proliferation and differentiation, and has insulin-like metabolic effects. The liver is the main organ producing IGF I in humans and rodents, and its production is
modulated by several factors, such as growth hormones, estrogen, and insulin, and also by fasting. The IGF type I receptor is structurally similar to the insulin receptor, a trans-membrane glycoprotein with an extracellular ligand binding domain and a cytoplasmic portion with tyrosine kinase activity. However, IGF type II receptors are identical to the cation independent mannose 6-phosphate receptor, which functions as a lysosomal enzyme targeting protein. In bone cells, the action of IGF I is regulated by various systemic and local factors, including growth hormone, PTH, vitamin D3, corticosteroids, TGFβ, IL-1, and platelet-derived growth factor (PDGF). It has been shown that IGF I, when added to PDL cells in culture, causes a dose-related increase in DNA synthesis. The evidence is also increasing regarding the role of IGF II in fibroblasts, where it influences both calcium influx and DNA synthesis. The evidence is also increasing regarding the role of IGF II in fibroblasts, where it influences both calcium influx and DNA synthesis. 

Each time mechanical damage to the periodontal vasculature is created by orthodontic force, platelets migrate from the blood vessels to the extravascular space. These platelets are a major source of growth factors for mesenchymal cells, in the form of PDGF. Originally, PDGF was isolated from platelets, but it was later found to be synthesized by various cell types. Two distinct types of PDGF receptors have been identified, the α receptor (which binds all 3 isoforms, PDGF AA, PDGF AB, and PDGF BB), and the β receptor (which binds to only PDGF BB). The 2 receptors are similar in structure, with an extracellular ligand-binding portion, a single transmembrane anchoring domain, and a highly conserved intracellular protein, tyrosine kinase. When PDGF binds to the extracellular portion, the receptor undergoes dimerization and autophosphorylation with activation of tyrosine kinases. The ligand-receptor complex is then internalized and degraded, leading to (1) activation of phospholipase A2-release of arachidonate acid, which, via cyclooxygenase and lipoxygenase activity, leads to formation of prostaglandins and leukotriens; (2) activation of phospholipase-Cy through a G-protein, with degradation of PIP2 and formation of IP3 and DAG; and (3) recruitment of substrate proteins to an oligomerized growth factor receptor, with increased tyrosine kinase activity. The proteins include PI3 kinase, Ras-GAP, and PLC-γ. Davidai et al and Sandy et al with different experiments, proposed this pathway to be important in mitogenesis in bone cells. They reached this conclusion by observation that inhibitors of tyrosine kinases can block PDGF-stimulated cell proliferation and receptor phosphorylation.

Connective tissue growth factor (CTGF) is another secreted protein that is associated with the ECM during anabolic bone remodeling. This signal molecule enhances vascular invasion, stimulates proliferation of osteoblast precursors, and promotes mineralization of new bone by osteoblasts. In alveolar bone, CTGF is localized in osteoblasts and osteocytes near the PDL. After 12 hours of experimental tooth movement, CTGF is expressed in osteoblasts and extends to osteocytes deep in the bone on both sides of the moving root.

**COLONY-STIMULATING FACTORS**

Colony-stimulating factors (CSF) include those related to granulocytes (G-CSF), macrophages (M-CSF), or to both cell types (GM-CSF), and might have particular implication in bone remodeling through osteoclast formation and thereby during tooth movement. These molecules are specific glycoproteins, which interact to regulate the production, maturation, and function of granulocytes and monocyte-macrophages. Fibroblasts and endothelial cells synthesize M-CSF. It was demonstrated by Kahn and Simmons that osteoclasts can form as a result of cultivating bone-marrow cells with M-CSF for 10 days. It has also been demonstrated that stimulation of fibroblasts with epidermal growth factor, PDGF, FGF, and IL-1 induce M-CSF expression by these cells. Takahashi et al reported that, in terms of potency, M-CSF is the most potent in stimulating bone-marrow cells to produce osteoclasts, followed by GM-CSF, IL-3, and G-CSF.

The above review of signal molecules that modulate various steps of tissue remodeling introduces the orthodontist to the complexity and minute details of events that appear to have major roles in this process. Prominent participants are products of the nervous, immune, and endocrine systems, but many locally produced growth factors and CSF appear also to participate actively in remodeling mineralized and non-mineralized connective tissues. Clinically, orthodontic patients might sense pain shortly after appliance activation. However, this feeling is just one of many reactions on the cellular and molecular levels that typifies orthodontic tissue remodeling.

**Genetic mechanisms**

Mechanical activation of bone cells is linked to many genes, which produce various enzymes, such as glutamate/aspartate transporter, inducible nitric oxide synthase, and prostaglandin G/H synthetase. In-situ hybridization under conditions of physiologic tooth movement in rats showed site-specific expression of mRNA for osteonectin, osteocalcin, and osteopontin. Osteoclast and osteoblast progenitor cells had positive signals for osteonectin and osteocalcin. Osteopontin was
expressed in osteoblasts and adjacent osteocytes along bone-resorbing surfaces. In response to orthodontic force, osteopontin mRNA was elevated in the tissue by 12 hours and this response was found to persist after 48 hours. It was proposed that at least 26 genes are involved in osteoclast differentiation and regulation, including tyrosine kinase gene, M-CSF, C-fos, Pu.1, and NF-kB (osteoclast formation), and C-tyrosine kinase and microphthalmia transcription factor in osteoclast activity. The role of the RANK-RANKL-OPG axis in bone remodeling was discussed in the previous section. Kanzaki et al observed an increase in RANKL mRNA expression in the PDL after exogenous PGE2 treatment and concluded that, in mechanically stressed PDL cells, RANKL up-regulation depended on PGE2. In addition, another gene, TREM-2 of TNF, has been implicated in the control of bone modeling and brain function. Wilde et al demonstrated periostin (a 90 kDa protein), showing a divergent expression pattern in PDL fibroblasts and osteoblastic cells in alveolar bone surfaces, even after 168 hours of tooth movement.

Pavlin and Gluhak-Heinrich used a mouse model to study the mechanically induced regulation of osteoblast- and cementoblast-associated genes. They demonstrated a defined temporal pattern of cell-specific gene regulation in periodontal osteoblasts, mechanically stimulated to differentiate and deposit bone matrix. According to these investigators, the primary responses to osteogenic loading are induction of differentiation and increased cell function, rather than an increase in cell numbers. They detected alkaline phosphatase and bone sialoprotein genes after 24 hours of treatment, followed by a concomitant stimulation of osteocalcin and collagen I between 24 and 48 hours, and deposition of osteoid after 72 hours. They reported that differential genetic responses to mechanical loading provide functional markers for a distinction between the cementoblast and osteoblast phenotypes.

Recent research has thus begun to unveil the identity of genes that control the synthesis of specific cellular and ECM components during tissue remodeling in response to mechanical loads. Continuous research in molecular genetics might identify additional genes that are activated by applied loads, leading ultimately to the development of new diagnostic, predictive, and therapeutic means in clinical orthodontics.

REFERENCES


3 Zhen Tan, Qing Zhao, Yangxi Chen. The mutual effects between orthodontic tooth movement and estrous cycle or estrogen. Biological Rhythm Research. 2010; 41(1): 75-81.


10 Alhashimi N. Frithiof L. Brudvik P and Bakhkiet M. Orthodontic movement induces high numbers of cells expressing interferon γ at mRNA and protein levels, J Interferon Cytokine Res 2000; 20: 7-12.


15 Katagiri Tand Takahashi N. Regulatory mechanisms of osteoblast and osteoclast differentiation, Oral Dis 2002; 8: 147-59.


18 Holdin CH. Miyazono K and ten Dijke, TGFβ signaling from cell membrane to nucleus through SMAD proteins, Nature 1997; 319: 511-14.

19 Shi Y. Structural insight on SMAD function in TGFβ signaling, Bioessays 2001; 23: 223-32.


29 Farndale RW, Sandy JR, Atkinson SJ, Pennington SR, Meghji S and Meikle MC. Parathyroid hormone and prostaglandin E2 stimulate both inositol phosphates and cyclic AMP accumulation on mouse osteoblast cultures, Biochem J 1988; 252: 263-68.


