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Molecular Biology of Orthodontic Tooth Movement

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Abstract

The application of orthodontic forces to correct mandibular and maxillary teeth irregularities through alveolar bone remodeling involves a series of coordinated and regulated molecular and cellular events in the periodontium i.e. periodontal ligament (PL), alveolar bone (AB), cementum, and gingiva. The PL and AB are the two important structures which actively participate in bone remodeling in response to mechanical forces. The fibroblasts, osteoblasts, osteocytes, osteoclasts, odontoblasts, cementoblasts, chondrocytes and immune cells are the major cell types which play an interactive role in the remodeling process. Activation of these cells result in the production of several pro-inflammatory cytokines, growth factors, colony- stimulating factors, transcription factors and other regulatory molecules which modulate cell growth, proliferation, migration, differentiation, gene expression and cell function. Recent it has been shown that the role of SOX 9 gene transcriptase, parathyroid hormone related peptide (PTHrP), Indian hedgehog (IHH) protein in orthodontic tooth movement orthopaedics is significant in understanding the molecular biology of orthodontic tooth movement orthopaedic forces in growth modification therapy. In this article, however, we review the major cellular and molecular sequence of events during orthodontic tooth movement, *per se*.

Keywords: Orthodontic tooth movement; ECM, Molecular; SOX 9; Il-1beta, osterix; Run-x 2; Bone cells; PL cells

Introduction

The periodontium consists of the periodontal ligament (PL), alveolar bone (AB), cementum and gingiva. The PL is a specialized matrix rich, mixed cellular/ fibro-connective tissues. It plays a pivotal role in signal transduction pathways, involving repair and remodeling of the PL, cementum and alveolar bone [1-4]. Homeobox protein MSX 2 acts a molecular defense mechanism for preventing ossification in ligament fibroblasts and prevents ankylosis of the tooth [5]. Fibroblasts, osteoblasts, osteocytes, osteoclasts, odontoblasts, cementoblasts, chondrocytes and immune cells are the major cell types involved in the remodeling process. Fibroblasts are the major group of cells found in the PL [6-8]. The PL contains primarily the Type I and Type III collagen fibers and the Type 1 is the dominant collagen [9,10]. The principal and oxytalan fibers are the predominant elastic fibers, which provide elasticity to the ligament during the tension related force on the ligament [11]. The PL extracellular matrix (ECM) contains a large quantity of glycoproteins, and proteoglycans (biglycans, decorins), fibromodulin, and fibronectin. These molecules perform multiple functions including cell migration and cell proliferation [12]. They also readily respond to the mechanical forces. Figure 1 presents a sagittal graphic view of the anatomical and vascular structures of the periodontium.

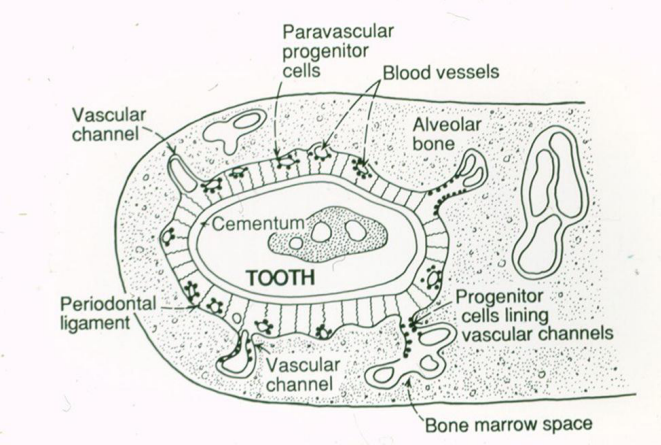

Figure 1: Shows the anatomical and cellular structures of the periodontium (By Dr. Lekic)

Orthodontic Tooth Movement and ECM Remodeling

The orthodontic tooth movement (OTM) exerts physical, biophysical and biochemical effects on the ECM and constituent cells of the periodontium and dental pulp [2,13-15]. Figure 2 shows the sequence of cellular and molecular events following the orthodontic tooth movement. The strain on the ECM

causes fluid displacement in alveolar bone canaliculi, PL vasodilatation, acute inflammation and inflammation-mediated nociceptive pain [16]. The fluid displacement leads to the physiological activation of osteocytes, osteoblasts, bone lining osteoblasts/osteoprogenitor cells as well as PL fibroblasts [17]. The nociceptic pain causes the PL neurons to secrete neuropeptides such as Substance P, calcitonin gene related peptide (CGRP) [18-21]. These peptides along with prostaglandin E-2 (PGE-2) and humoral factors cause the dilatation of PL capillaries. This leads to the release of immune competent cells from the capillaries [15]. Migration of these cells is mediated by the chemotactic factors and vascular endothelial growth factor (VEGF) secreted by endothelial cells and osteoblasts. VEGF is an essential mediator for bone angiogenesis and in bone development [22]. The ECM remodeling is followed by cytoskeletal re-organization in osteoblastic cells [23]. Cytoskeletal re-organization leads to phosphorylation of cellular proteins including extracellular signal-regulated kinases (ERK) [24]. This triggers signal transduction via integrins/fibronectin/kinase- pathway [25-28]. Intercellular communication occurs through gap junction proteins (connexions). Several matrix metalloproteinase (MMP) particularly 9, 3, 13, 1, 8 are increased on the pressure side and an active collagen remodeling occurs. Prostaglandin-2 (PGE 2) and COX 2 mRNA are also up regulated on the compression side [29,30]. M-CSF, a secretory product of osteoblasts regulates the differentiation of osteoclast precursors to mature osteoclasts [31]. Recent work from University of Honkong on the cell biology of tooth movement and the role of transcription factor Sox-9 and parathyroid hormone related protein (PTHrp) and Indian Hedgehog protein are very significant in understanding the molecular biology of orthodontic tooth movement force transduction [32]. Recent evidence shows that SOX 9 directly regulates the Type II collagen gene [31]. It is a chondogenic transcription factor and a target of signaling by the PTHrP in the growth plate of endochondral bone. SOX-9 also prevents the conversion of proliferating chondrocytes into hypertrophic chondrocytes [33]. Indian Hedgehog (IHH) is protein involved in chondrocyte differentiation, proliferation and maturation especially during endochondral ossification. It regulates its effects by feedback control of PTHrP. PTHrP regulates extracellular matrix gene expression in cementoblasts and inhibits cementoblst-mediated mineralization in vitro [34], all mechanisms of interest and importance in orthopaedic growth modification.

Role of Cytokines, Growth Factors and Transcription Factors

The strain and stretch effects caused by orthodontic forces induce PL fibroblasts, osteocytes, osteoblasts and osteoclasts lead to the production of a number of messenger molecules as shown in Figure 3. Periodontal ligament and PL immune cells produce pro-inflammatory cytokines (IL-1 beta, Il-6, IL-8, Il-12, IL-13 TNF alpha) and anti-inflammatory cytokine IL-10 [35-37]. These molecules modulate cell growth, proliferation, cell migration, differentiation, gene expression and cell specific functions [15,38,39]. IL-1 β is considered an important cytokine in tooth movement due to its pleotropic effects. Tumour Necrosis Factor alpha (TNF α), is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells including bone resorption by osteoclasts. RANKL is a member of the tumor necrosis factor (TNF) cytokine family which is a ligand for osteoprotegerin (OPG) and functions as a key factor for osteoclast differentiation and activation. The orthodontic tooth movement activates osteoblasts. In response, osteoblasts produce in a spatial manner a number of key molecules including bone morphogenetic proteins (BMPs), macrophage colony stimulating factor (M-CSF), receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), transcription factors (osterix, Run X-2), heat shock protein (HSP), fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor beta (TGF beta), insulin growth factor (IGF). BMP-2 and BMP-7 are involved in osteoblast differentiation. Each molecule has a specific role to play in the complex signaling network. M-CSF is stimulated by PTH; induces osteoclast differentiation. Osterix is a zinc finger- containing transcription factor, induces osteogenic gene expression in primary human mesenchymal cells [40]. Osteoblast differentiation is also induced by hedgehogs and core binding transcription factor alpha-1 (cbfa1) [41]. BMP-2 regulates osterix through msx-2 and nunx-2 during osteoblast differentiation [42]. The Runt related transcription factor-2 (Runx-2) mediates regulation of osterix and it helps in osteoblasts differentiation [43]. BMP-2 also induces dental follicle cells to differentiate toward a cementoblast/osteoblast phenotype [44]. Osteocytes produce sclerostin, phosphate regulating endopeptidase homolog, X-linked. (PHEX), dentin matrix phosphoprotein-1(DMP-1), c-fos, TGF beta, matrix extracellular phosphoglycoprotein (MEPE), hypoxia induced factor (HIF-1), NO, PGE-2, IGF, c-fos. HIF-1 and c-fos are associated with hypoxia and angiogenesis [45]. Ischemia and hypoxia occur on the pressure side as a result of reduced blood supply. HIF-1 inhibits Wnt signaling in osteoblasts, thus inhibiting osteoblast differentiation as Wnt signaling is responsible for osteoblast differentiation. MEPE produced by osteoblasts are involved in integrin recognition [46]. Integrins play vital role in cell signaling mechanism. Epithelial cells produce integrins, cytokines, vascular endothelial growth factor (VEGF). Integrins are also produced by osteoblasts. VEGF is produced by vascular endothelial cells, osteoblasts, osteoclasts and fibroblasts [47]. PHEX and DMP 1 regulate fibroblast growth factor (Fgf23) [14]. Prostaglandin E-2 is produced by platelets, endothelium, and mast cells and also is liberated as breakdown products of membrane phospholipids during orthodontic tooth movement and is involved in inflammation, vasodilatation and pain. It stimulates osteoblasts that releases factor that stimulate bone resorption by osteoclasts. MMPs are secreted by fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. They are responsible for the tissue remodeling and degradation of extracellular matrix substances including collagens, elastins, gelatin, matrix glycoproteins and proteoglycans. They are regulated by hormones, growth factors, and cytokines. Osteoclasts produce chemokines (CCR2, CCR5), and epidermal growth factor (EGFR). All cellular activities in the periodontium are regulated by multiple molecules and mechanisms. The key molecules are: cytokines, BMP,

TIMPS, TGF beta, NO, sclerostin, noggin, PTH, integrins and DNA binding regulatory proteins. The major signaling systems include Erk1/2, NFkβ, NO, RANK/RANKL/OPG, P2X7 Wnt, & Notch. The basic functions of these molecules and pathways are to activate and regulate cell growth, proliferation, migration, differentiation, gene expression and cell functions and remodel ECM, PL, and alveolar bone.

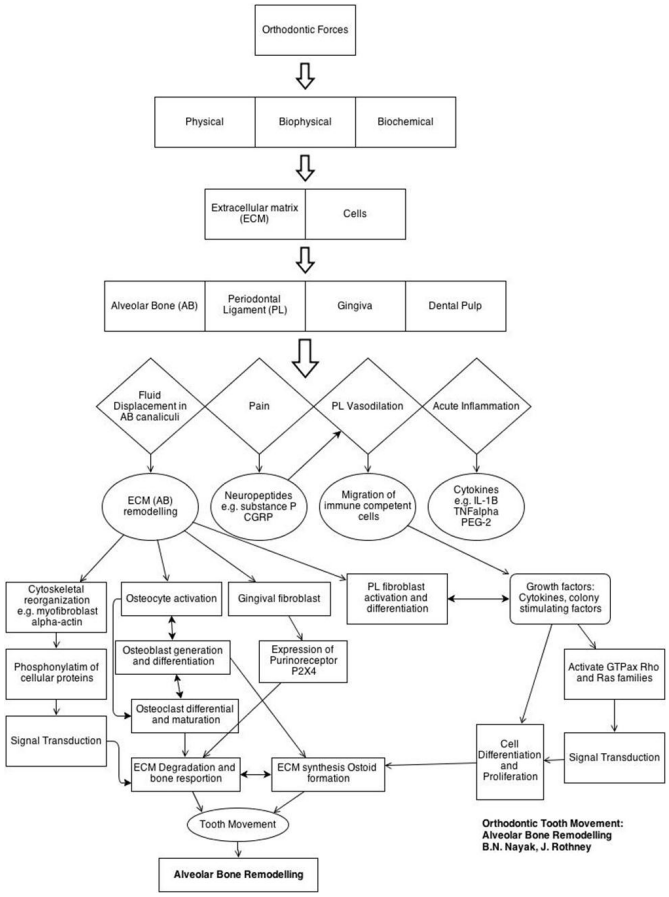

Figure 2: Orthodontic tooth movement & sequence of molecular events

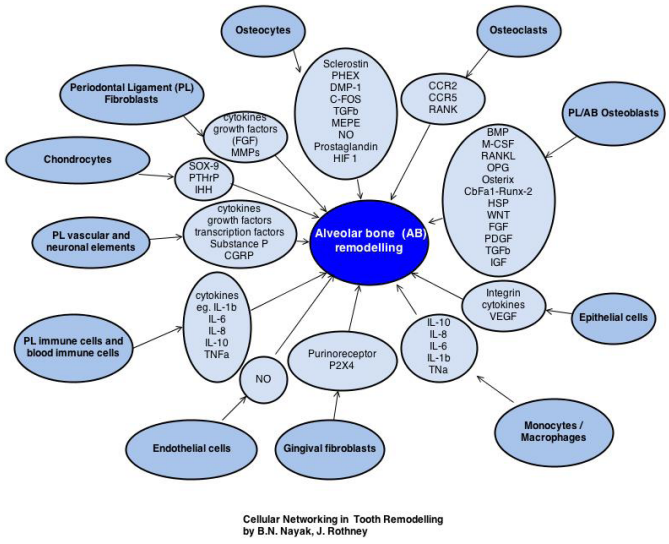

Figure 3: Cellular networking in tooth remodeling

Role of growth factors

Bone morphogenetic proteins are a group of growth factors with cytokine properties [48]. BMP 2 and BMP 7 are produced by osteoblasts and are involved in osteoblast differentiation. BMP 2 also plays role in cementoblast differentiation [44]. Osteopontin (OPN) is a multifunctional protein, biosynthesized by fibroblasts, osteoblasts, osteocytes, odontoblasts, bone marrow cells, and hypertrophic chondrocytes. Periodontal ligament show an elevation in OPN on the tension side of the PL [49].

Role of MMPs

Matrix metalloproteinases (MMPs) are large family of calcium- dependent Zinc-containing endopeptidases which are responsible for the tissue remodeling and degradation of extracellular matrix proteins. MMPs are key enzymes in the remodeling of PL [50].

P2X4 receptors

P2X4, an ATP receptor subtypes expressed on immune, neural cells and gingival fibroblasts and involved in the regulation of ATP dependent signaling. It is up regulated in gingival fibroblasts after periodontal surgery [51].

Role of bone cells

Osteoblasts are one of the active groups of cells in orthodontic tooth movement. They produce bone morphogenetic proteins (BMPs), macrophage- colony stimulating factors (M-CSF), receptor activator of nuclear factor kappa-B ligand (RANKL) RANKL, OPG, HSP, FGF, PDGF, TGF beta, IGF, IL-1 beta, IL-6, NFkB and transcription factors SOX 9, osterix, Cbfa1/runx-2, Wnt. Runx-2 expression leads to enhanced production of OPN, Bone sialoprotein (BSP), Collagen 1, alkaline phosphatase (ALP). Osteocytes are mechanosensory cells. Osteoblasts and PL fibroblasts are mechanoresponsive cells. These cells and their precursors play important role in PL and alveolar bone remodeling. They are multi-processed cells with relatively thin cytoplasm, connected to each other between lacunae and alveolar bone canaliculi and also in contact with bone lining osteoblasts and stem cells. They are the chief mechanosensory cells in the peridontium in response to orthodontic tooth movement. Osteocyte produces sclerostin, PHEX, DMP-1, c-fos, TGF beta, MEPE, NO, prostaglandins, HIF 1, IGF [52]. PL fibroblasts produce a number of proinflammatory (IL-1 beta, IL-6, IL-8, TNF alpha) and anti-inflammatory (IL-10) and ECM proteins including Col 1. OPN is a multifunctional molecule [53] which contains an Arg-Gly-Asp (RGD) motif that is known to promote osteoclast attachment through integrins & CD4 [54-56]. Osteoclasts produce RANK, CCR2, CCR5. Osteoclasts differentiation is inhibited by IL-12, IL-18, IL-33, IFN. Osteoclasts are activated by TNF alpha, IL-1 and IL-17. Osteoclasts differentiation is regulated by PTH, calcitonin, IL-6, OPG and RANKL.

Role of PL fibroblasts

These are versatile group of cells. PL fibroblasts produce BMPs, cytokines such as IL-1 beta, Il-8, TNF alpha, transcription factor osterix, ALP, OPN, BSP, SOX 9. PL fibroblasts produce a

number of pro- and anti-inflammatory cytokines (Il-1 beta, Il-6, Il-8, Il-10, TNF alpha, TGF beta, EGF, MMPs) indicating the role TGF beta has in cell proliferation and differentiation.

Role of monocytes and macrophages

Activation of monocytes/macrophages produces several pro- and anti-inflammatory cytokines such as IL-1 beta, IL-6, Il-8, IL-10 TNF alpha.

Role of membrane phospholipids

Tooth movement causes cellular damage resulting in the production of many membrane phospholipids derived messenger molecules such as lipoxins, prostaglandins and leukotrienes. These molecules arise from the arachidonic acid (AA) pathway. AA is an unsaturated fatty acid, a normal constituent of membrane phospholipids, and is released by action from phospholipase A2. Notably, prostaglandins arise from a cyclic endoperoxide generated by enzyme system PG synthesis (e.g. cyclooxygenase).

Role of nitric oxide

Nitric oxide (NO) is produced in endothelial cells during orthodontic tooth movement and is involved in vasorelaxation, platelet aggregation and cardiovascular homeostasis. NO induces relaxation of smooth muscle cells in blood vessels in the PL, can stimulate guanylate cyclase leading to generation of the second messengers. Expression of nitric oxide synthases in orthodontic tooth movement has been reported [57,58]. Production of nitric oxide and prostaglandin E (2) by primary bone cells is shear stress dependent.

Role of chemokines

Chemokines constitute a family of chemoattractant cytokines and are subdivided into four families on the basis of the number and spacing of the conserved cysteine residues in the N-terminus of the protein. Chemokines play a major role in selectively recruiting monocytes, neutrophils, and lymphocytes, as well as in inducing chemotaxis through the activation of G-protein-coupled receptors. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages. Migration of monocytes from the blood stream across the vascular endothelium is required for routine immunological surveillance of tissues, as well as in response to inflammation. Chemokines are upregulated during orthodontic tooth movement [59].

Role of transcription factors

Runx2 and osterix (Osx) transcription factors are required for the expression of OPN. BSP is a mineralized tissue-specific non-collagenous protein produced by osteoblasts, plays potential role in the initial mineralization of bone, dentin and cementum.

Pressure: Tension Related Effects

When the periodontal ligament is stretched, bone apposition occurs on the tension side due to the increased activity of osteoblasts along with other local and systemic hematopoietic cells and bone resorption occurs on the compressed side by the multinucleated osteoclasts. The osteoblasts are activated and induced to express BSP mRNA, which is involved in bone remodeling. Differentiation and functions of osteoclasts are regulated by osteoblasts derived RANKL. Orthodontic tooth movement also induces the proliferation of epithelial rests of Malassez at the root of the tooth. Recently it has been reported that insulin-like growth factor-1(IGF-1), its receptor (IGF-IR), and insulin receptor substrate (IRS 1) are expressed as an early reaction of PL cells to experimental tooth movement in the rat model.

RANK RANKL/OPG pathway

The RANK/RANKL/OPG signaling pathway is essential for oesteoclastogenesis. This signaling pathway is inhibited by the binding of OPG to RANKL. Osteoprotegerin (OPG) is a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL). By binding RANKL, OPG inhibits nuclear kappa B (NF-κB) [52]. Osteoprotegerin levels are influenced by voltage-dependent calcium channels Cav1.2. OPG can reduce the production of osteoclasts by inhibiting the differentiation of osteoclast precursors.

Regulation

The biological activities in the peridontium during orthodontic tooth movement are regulated by multiple signaling molecules and pathways which include ERk1/2, NFkβ, P2X7, WNT, NOTCH, BMP, NOGGIN, NO, TGF beta, and p38 MAPK, ERK/JNK [60]. Some of these signaling systems operate in a temporo-spatial manner. It has been shown that mechanical signals are transmitted into the nucleus by ERK/JNK signaling pathways and then stimulate Collagen I expression through AP-1 activation in force-exposed human periodontal ligament fibroblasts [61]. BMPs which are produced by osteoblasts, regulate osteoblast differentiation. The process is regulated by a substance like noggin. MMPs which are produced by osteoblasts are involved in collagen digestion and osteoclastogenesis. The MMPs activities are regulated by tissue inhibitor TIMPs and also and also by hormones, growth factors and cytokines. Sclerostin, a product of osteocyte negatively regulate several members of BMPs and is inhibited by PTH and mechanical loading. Sclerostin by binding to LRP 5/6 receptors inhibits Wnt signaling pathway leading to decreased bone formation. Runx-2, a transcription factor produced by osteoblast induces differentiation of osteoblasts and also modulates BMPs. Integrins transmembrane receptors attach with other cells or ECM induces signaling pathways by changing intracellular Ca^{2+} regulate inositol lipid turn over & phosphorylation of intracellular proteins. MLO-Y4, a product of osteocyte stimulates surface lining osteoblasts. MAPK ERK ½, MAPK JNK, MAPK p38 and MAPK ERK -5 induce cell differentiation and proliferation. IL-8 induces IL-1 beta. IL-1 beta induces TNF alpha. Ischemia and hypoxia resulting from ECM remodeling induce osteocytes to produce HIF1. Bone resorption occurs through RANK/RANKL/OPG pathway. IL-10 produced by monocytes up regulates OPG, down regulate RANKL. TNF alpha RI (p55) stimulates osteoclastogenesis, while TNF alpha RII suppresses osteoclastogenesis. Heat shock protein produced by osteoblasts prevents osteoblast cell death by TNF alpha. TGF beta

induces fibroblasts to myofibroblast. Myofibroblasts express alpha SMA. TGF beta also inhibits osteoclast precursors. Mechanical stress transiently activates MAP kinases which activate AP-1, NFkB, c-fos, c-jun. These activations lead to cell differentiation, proliferation and activation. Fluid stress increases NO, PGE-2, IL-8, down regulates ALK, MIP-1 alpha mRNA. Mechanical stress transiently activates. Osteocytes through signaling mechanism activate osteocytes which then express RANKL and secrete macrophage colony-stimulating factors (M-CSF). RANKL is the ligand for NFkB. M-CSF stimulates macrophages to secrete pro-inflammatory cytokines such as TNF alpha.

Conclusion

The periodontium undergoes a series of coordinated and regulated cellular and molecular events following application of orthodontic forces of physiological magnitude. The PL and AB actively involved in bone remodeling. Osteocytes, osteoblasts, PL fibroblasts, osteoclasts, chondrocytes and immune cells are the principal cell types responsible for producing a number of cytokines, growth factors, and transcription factors and other regulatory molecules which modulate cell proliferation, differentiation, gene expression and cell functions. The ECM molecules as well as osteocytes, osteoblasts and PL fibroblasts show a remarkable response to the orthodontic forces. Recent evidence that SOX-9 gene, PTHrP and IHH play a major role in orthodontic tooth movement is of particular interest.

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