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Molecular basis of orthodontic tooth movement

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Certification of the Supervisor

I certify that this project entitled **"Molecular basis of orthodontic tooth movement"** was prepared by the fifth-year student **Abbas abdulhussen** under my supervision at the College of Dentistry/University of Baghdad in partial fulfilment of the graduation requirements for the Bachelor Degree in Dentistry.

Supervisor's name: lecturer Dr.Hadeel adil

Date:2023

Dedection

This research is dedicated to my supervisor **Dr. Hadeel adel**,my family and my friends for

their unfailing and selfless love, support, and care,

for which I will forever be grateful

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Aspartate aminotransferase (AST)

Arachidonic acid (AA)

Dentine sialophosphoprotein (DSPP).

Dentine matrix protein 1 (DMP1)

Endothelial nitric oxide synthase (eNOS)

Epidermal growth factor (EGFR).

Fibroblast growth factor (FGF)

GFs matrix metalloproteinases (MMPs).

Heat shock protein (HSP)

Inducible nitric oxide synthase (iNOS)

Insulin growth factor (IGF)

Lactate dehydrogenase (LDH)

Myeloperoxidase (MPO)

Macrophage colony stimulating factor (M-CSF)

Matrix metalloproteinase-1 (MMP-1)

Nitric oxide (NO)

Osteoprotegerin(OPG)

Osterix (Osx)

Orthodontic tooth movement(OTM)

Polymorphonuclear neutrophil (PMN)

Platelet derived growth factor (PDGF),

Prostaglandin E2 (PGE2)

Receptor activator of nuclear factor kappa Beta (RANK)

Receptor activator of nuclear factor kappa Beta ligand(RANKL)

Tartrate resistant acid phosphatase (TRAP)

Transforming growth factor beta (TGF beta),

Tumour Necrosis Factor alpha (TNF α),

INTRODUCTION

The success of orthodontic treatment is influenced by a number of factors, including periodontal health, oral hygiene, and orthodontic forces **(Cardaropoli and Gaveglio, 2007).**

The development of new methods to accelerate orthodontic tooth movement (OTM) has been sought by clinicians as a way to shorten treatment times, reduce adverse effects such as pain, discomfort, dental caries, and periodontal diseases, and minimize iatrogenic damages such as root resorption and the subsequent development of nonvital teeth. Tooth movement induced by orthodontic force application is characterised by remodelling changes in the dental and periodontal tissues **(Vinod and Ze'ev, 2006)**.

Two interrelated processes involved in OTM are

(1) deflection, or bending, of the alveolar bone and

(2) remodelling of the periodontal tissues, including the dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva.

Various cell-signalling pathways are activated, which ultimately stimulate PDL turnover, as well as localised bone resorption and bone deposition **(Dolce *et al*., 2002)**.

The sequence of events following OTM can be characterized using suitable biomarkers. A biomarker is a substance that is measured and evaluated objectively as an indicator of normal biologic processes, pathogenic processes, or

pharmacologic responses to a therapeutic intervention **(Theodosia *et al*., 2009)**. In investigating biomarkers, the rate, amount, and activity of the released substances not only reflect the activity of individual cells but also indicate the metabolic activity in the involved tissues or organs **(Mario *et al*., 2005)**.

This research will discuss the tissue changes that are involved during OTM such as at compression region (involving osteoblasts), tension region (involving osteoclasts), and dental root and pulp tissues. The involvement of stem cells and their development towards osteoblasts and osteoclasts during orthodontic treatment also will be discussed in this research.

Aim of study

Our primary aim is to illustrate the molecular mechanism of orthodontic tooth movement. The cellular and tissue changes associated with orthodontic tooth movement are our objectives.

Chapter 1 Review of literature

1.1 Orthodontic tooth movement

in 1962 Burstone suggested three phases of tooth movement (OTM) **(Burstone *et al*., 1962)**. They are: Initial phase, Lag phase and Post lag phase.

Initial phase is the movement of the tooth within the bony socket. It occurs immediately after the application of force to tooth. The movement is rapid due to the displacement of tooth in periodontal space. The time frame of the initial phase usually occurs between twenty-four hours to two days **(Burstone *et al*., 1962)**. Due to the force applied on the tooth there is a compression and stretching of periodontal ligament which in turns causes extravasation of vessels, chemo-attraction of inflammatory cells and recruitment of osteoblast and osteoclast progenitors (Figure 1). **(Nowak-Solinska E, *et al*,2012)**

After the initial phase, there is a **lag phase** in which the movement is minimal or sometimes no movement at all. The reason for this phase is the hyalinization of compressed periodontal ligament. The movement will not take place until the necrosed tissue is removed by the cells **(Kashyap, 2016)**.

In the lag phase the tooth movement stops for twenty to thirty days and during this time frame all the necrotic tissue is removed along with the resorption of adjacent bone marrow. The necrotic tissue from the compressed bone and compressed periodontal ligament sites are removed by macrophages, foreign body giant cells and osteoclast cells**(Kashyap, 2016)**..

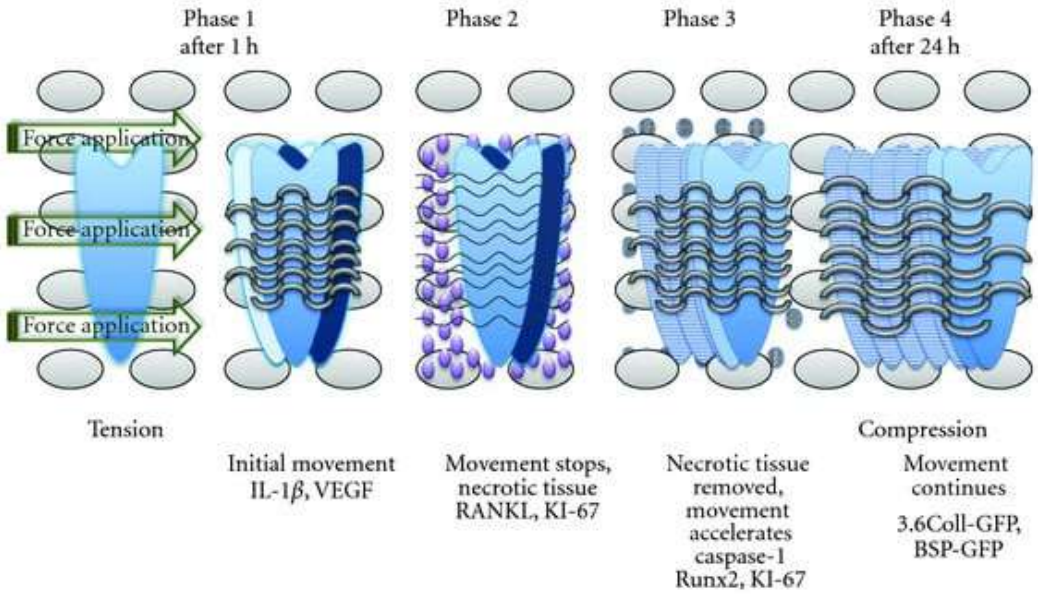

Figure 1 Phases of OTM **(Nowak-Solinska E, *et al*,2012)**

The third phase is the **post lag phase** in which the movement of tooth gradually or suddenly increases and is usually seen after forty days after the initial force application. It has been hypothesized that during displacement of tooth, acontinuous development and removal of necrotic tissue occurs (Figure 2)

(Yasunobu *et al*., 2010).

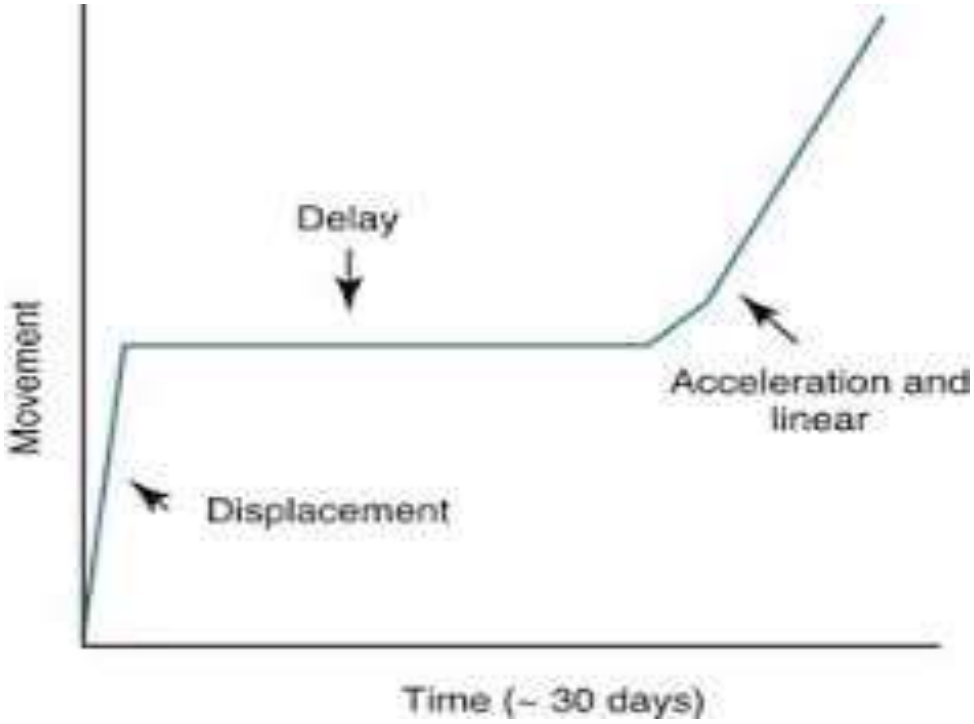

Figure 2 The biological mechanism of orthodontic tooth movement. **(Lekic PC, *et al.*, 2005)**

1.2 Tissue and cell changes during orthodontic tooth movement

The periodontal tissues primarily affected by orthodontic forces can be divided histologically into two main regions: the compression region and tension region. Other regions that are affected are the dental root and pulp **(Domon *et al.*, 1999)**.

The early response of periodontal tissues to mechanical stress involves several metabolic changes that enable tooth movement. A slight change in the thickness of the PDL occurred after 1 hour of orthodontic force loading, while more significant changes were seen after 6 hours **(Lekic PC. *et al.*, 2005)**.

1.2.1 Compression Region

The compression region is an area that is pressed by the orthodontic appliance in the direction of the force. Compression results in the deformation of

blood vessels and disarrangement of tissues surrounding teeth. Subsequently Figure (3), blood flow and periodontal tissue changes may adapt to the compression force. Metabolic changes can occur to the cells of the periodontal ligament as a result of hypoxia and decreased nutrient levels **(Spela *et al*., 2008)**.

In hypoxic conditions, cells will rely on anaerobic glycolysis. Many enzymes involved in an anaerobic metabolism can be potential markers. Lactate dehydrogenase is an example of a molecule that accumulates during anaerobic metabolism **(Spela *et al*., 2008)**.

Cells that adapt via metabolic changes will continue to live and cells that cannot adapt to the ischaemic condition will die, the dead cell will lyse, releasing all of its contents to the milieu and subsequently causing the activation of local inflammatory processes **(Lekic PC*et al*., 2005)**.

Mechanical forces often cause hyalinisation leading to necrosis in the PDL and lead to bone resorption. Hyalinisation occurs as cell-free areas of the PDL (Figure 3), in which the normal tissue architecture and staining characteristics of collagen in the processed histologic material have been lost. Distortions in the normal periodontal fibre arrangement were observed **(Philip *et al*., 2009)**.

Macrophages are ultimately responsible for removing the hyalinised tissues.

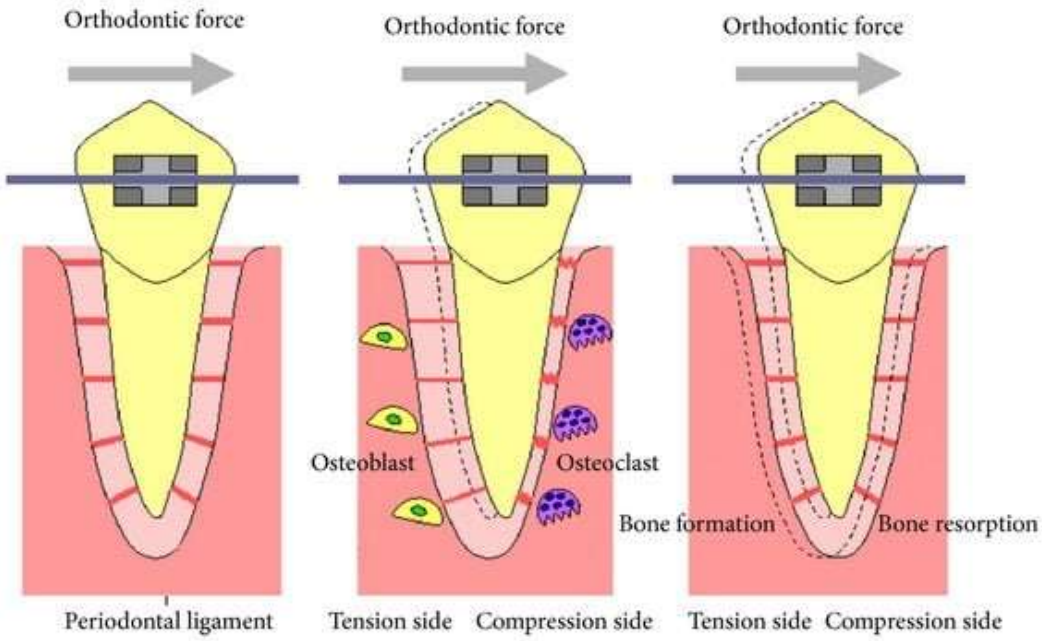

Figure 3 Tissue and cell changes during OTM, compression region and tension region. **(Kim HJ, *et al.* 2007)**

Alveolar bone resorption occurs at the compression areas during tooth movement. Bone resorption occurs through osteoclastic activity by osteoclast thus creating cavity in bone known as lacunae that later will be filled in by osteoblast cells to cover the cavity. Two processes involved in bone resorption are the solubilisation of minerals and the degradation of the organ matrix, which mainly consists of type I collagen,These processes are driven by proteolytic enzymes and, in particular, matrix metalloproteinases and lysosomal cysteine proteinases **(Yasunobu *et al.*, 2010)**.

According to the concept of tissue response after OTM, bone repair at the compression region only occurs when the magnitude of the force decreases.

1.2.2 Tension Region

In the tension region, new bone is formed as a result of forces applied by braces (also known as Dental braces , orthodontic cases, or cases are devices used in orthodontics that align and straighten teeth and help position them with regard to a person's bite, while also aiming to improve dental health.) during orthodontic treatment. Osteoblasts are differentiated from the local precursor cells, that is, mesenchymal stem cells. Mature osteoblasts form the osteoids and the mineralisation processes follow **(Domon *et al*., 1999)**.

In addition, endothelial nitric oxide synthase (eNOS) was shown to mediate the bone formation in the tension area (Figure 3) **(Spela *et al*., 2008)**, which in turn suggests that eNOS could be useful markers for osteoblastic activity. Enzyme profiles have also been investigated in relation to alveolar bone formation at tension sites. Another biochemical marker that may be useful during osteoblastic activity is alkaline phosphatase (ALP) (is an enzyme that's found throughout the body that comes from liver and bones,) **(Tan *et al*., 2009)**.

1.2.3 Dental Root and Pulp

One adverse effect caused by orthodontic treatment is root resorption, which is a common iatrogenic consequence in the field of orthodontics and may start during the early stages of orthodontic treatment **(Philip Brooks *et al*., 2009)**. Irreversible root resorption is caused by excessive forces or decreased resistance to normal forces. Roots do not shorten naturally with age, unless forces overcompress the PDL **(Baloul SS *et al*., 2011)** .

Some odontoclasts reside in root resorption site indicating that odontoclasts play central roles in root **(Yasunobu *et al*., 2010)**.

. Study on markers involved during odontoclast activity indicates that they can be potential markers for root resorption activity**(Domon *et al*., 1999)**.

Biomechanical treatment factors such as magnitude, duration, direction, and type of force (e.g., intermittent, interrupted, and continuous) can have an impact on root resorption ,However, the relationship between the amount of tooth movement and root resorption is less clear , and other unknown factors may influence the extent and depth of the root resorption **(Baloul SS *et al*: 2011).**

In rats, matrix metalloproteinase-1 (MMP-1) and cathepsin K are important in root resorption during tooth movement because they degrade However, it is still difficult to find precise biomarkers for root resorption or nonvital teeth because osteoclasts are also activated. Therefore, a conventional radiograph is still a cheap, effective, and important way to monitor root resorption. However, it is not an adequate tool in the diagnosis of apical shortening, lateral or cervical root gaps, enlargement of root canals, and external root radiolucencies in early stages. It also has the limitation of being a 2-dimensional image **(Aggarwal BB: 2000),** In contrast, computed tomography can also be used to evaluate root resorption using a 3-dimensional approach , However, the accuracy of this approach in determining root resorption warrants further evaluation.

1.3 Markers for orthodontic tooth movement

Applying orthodontic forces to teeth will ultimately result in movement. The main phenomena, both before and after tooth movement, are alveolar bone remodelling, tissue inflammation, and root resorption. Each of these events can potentially be detected using suitable markers**(Yano S *et al* .,2005).**

1.3.1 Markers of Alveolar Bone Remodelling

As orthodontic forces are applied to teeth, the compression region shows an elevation in osteoclastic activity. Meanwhile, in the tension region, osteoblasts begin to proliferate and mineralise the extracellular matrix. This orchestra results in alveolar bone remodelling **(Domon *et al*., 1999)**.

Chemokines may contribute to differential bone remodelling in response to orthodontic forces through the establishment of distinct microenvironments in the sites of both compression and tension .**(George A, Evans , 2009)**The principal trigger for OTM is most likely the strain experienced by the PDL cells, bone-related cells, and the extracellular matrix. This strain leads to changes in gene expression in the cells via interactions between the cells and the extracellular matrix **(Dolce *et al*., 2002).**

One of the examples are matrix metalloproteinases (MMPs). MMPs break down the extracellular matrix and are important in bone remodelling. Compression induces an increase in MMP-1 protein levels after 1 hour. However, the increase lasted for 2 hours and subsequently disappeared. Tension led to significantly increased levels of MMP-1 protein after just 1 hour of force application and also subsequently disappeared **(Aggarwal ,2000).**

MMP-2 protein was induced by compression and increased significantly in a time-dependent fashion, reaching a peak after 8 hours of force application. On the tension side, MMP-2 was significantly increased after 1 hour but gradually returned to basal levels within 8 hours **(Aggarwal , 2000).**This result indicates that MMP-2 could be used during very early stages of orthodontic treatment as a marker for active tooth movement.

1.3.2 Bone Formation Marker

Bone formation is primarily due to osteoblastic activities. Therefore, bone formation markers are usually osteoblastic enzymes or byproducts of bone formation such as type I procollagen. Type I procollagen was secreted by osteoblast cells **(Lam *et al* 2000).**

Bone formation can also be promoted by GFs via their interaction with specific surface receptors on osteoblasts, thereby stimulating insulin-like GF-1. Insulin-like GF-1 is a primary mediator of the effects of growth hormones that have growth-promoting effects on bone, in addition to regulate cell growth and development. Other studies have found that Msx1 and Msx2 are potential regulators of bone formation**(Teitelbaum and Ross 2003).**

Mechanical forces in orthodontic treatment cause the physical distortion of PDL and alveolar bone cells. They can also trigger a multilevel cascade of signal transduction pathways, such as the prostaglandin E2 (PGE_2) pathway, that initiate structural and functional changes in extracellular, cell membrane, and cytoskeletal proteins **(Burstone *et al*., 1962)**. Subsequent changes in cytoskeletal protein structure and function lead to the creation of new cells and bone matrix formation **(Teitelbaum and Ross ,2003).**

1.3.3 Bone Resorption Marker

Osteoclastic cells that are involved in bone resorption are specialised multinucleated giant cells that originate from haematopoietic stem cells. The earliest bone resorption marker is the interleukin-1 beta (IL-1β) **(Burstone *et al*., 1962)**. PGE_2, interleukin-6 (IL-6), and other inflammatory cytokines can also facilitate osteoclastic bone resorption processes **(Yano S *et al* .,2005).**

These proteins regulate osteoclastic activity through activation of the nuclear factor kappa B (RANK) and of the nuclear factor kappa B ligand (RANKL). Osteoblastic cells also control osteoclastic processes by synthesizing RANKL to promote more osteoclastic differentiation **(Bakker and Soejima K ,2001)**

On the other hand, the activation of the OPG gene, also through gene transfer to periodontal tissues, managed to neutralize RANKL activity and hence inhibits osteoclastogenesis and eventually OTM. There is an indication that the activation of the OPG gene inhibits the OTM process.

The enzyme assay of acid phosphatase activity in saliva was also introduced as a method used to measure biomarkers of OTM. On the basis of the enzymatic profile activity of lactate dehydrogenase, tartrate resistant acid phosphatase (TRAP) and ALP in mixed saliva ,suggested the reactivation of orthodontic braces from 30 days to 25 days so that the treatment course will be decreased by approximately 17% **(Ahuja SS, *et al.* 2003).**

Other biomarkers of early OTM were investigated in a rat model using a split-mouth design at 3 and 24 hours after appliance insertion. The spatial expression patterns of KI-67, RANKL, and Runx2 during OTM were mapped by using immunohistochemical staining**(Teitelbaum and Ross,2003).**

Nitric oxide (NO) is an important regulator of bone responses to mechanical stress and is produced through the activity of constitutive endothelial nitric oxide synthase (eNOS) or inducible nitric oxide synthase (iNOS). NO mediates adaptive bone formation, protects osteocytes against apoptosis and mediates osteoclastic activity. High levels of NO reduce osteoclastic activity, while the inhibition of NO production increases osteoclastogenesis and

osteoclastic activity **(Spela *et al*., 2008)**. Osteocalcin is the most abundant noncollagenous matrix protein found in bone. It is expressed by highly differentiated osteoblasts and is incorporated into the bony matrix. Smaller osteocalcin fragments are thought to be a degradation products of bone matrix, which suggests its potential as a bone resorption marker **(Bakker and Soejima K.,2001)**

1.3.4 Markers of Inflammatory Processes

The host response to orthodontic forces has been described as aseptic and transitory inflammation. Among the substances investigated are lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), which are inflammatory biomarkers found outside cells during necrosis. Increased levels of lactate dehydrogenase and aspartate aminotransferase were detected in human GCF samples obtained during OTM. However, increased levels of lactate dehydrogenase in whole saliva can be associated with periodontal disease as well, especially with the presence of calculus and periodontal pockets greater than 5mm. Aspartate aminotransferase is also found in periodontitis **(Rhee, *et al,* 2009).**

Myeloperoxidase (MPO) is an enzyme found in polymorphonuclear neutrophil (PMN) granules and can be used to estimate the number of PMN granules in tissues. [24] Mean MPO activity was increased in both the GCF and saliva of orthodontic patients at 2 hours after appliance activation. MPO might be a good biomarker to assess inflammation in orthodontic movement **(Ahuja SS *et al.* 2003).**

1.3.5 Markers of Root Resorption

Root resorption is either a physiological or pathological condition associated with tooth structure loss and is caused by osteoclastic cells. Orthodontic treatment invariably results in permanent root resorption. The resorptive process starts just below the gingival epithelial attachment of the tooth, extending apically and/or coronally along the root dentin. Usually **(Bakker and Soejima K.,2001)**even in advanced cases, there is no pulpal involvement because of the protective nature of the predentine layer, which explains the asymptomatic nature of this type of resorption. However, the very thin dentin layer that remains is at risk of perforation during the removal of the existing granulation tissue and may necessitate root canal treatment. The often delayed nature of the resorptive process is even more difficult to explain. According to one theory, there is a decrease in the ratio of organic to inorganic cementum cells **(Megat Abdul Wahab *et al*,2008).**

Dentine consists of noncollagenous proteins such as DMP1 (dentine matrix protein 1), dentine phosphoprotein (DPP), and dentine sialoprotein (DSP). DPP and DSP are products of mRNA transcription and are portions of one expressed protein known as dentine sialophosphoprotein (DSPP). Examination of patients undergoing active orthodontic treatment showed elevated levels of DPP relative to the control group **(Ahuja SS *et al*. 2003).**

The peripheral nervous system in the tooth pulp and periodontium contributes to the development of both acute and chronic inflammatory processes via the release of substance P (SP). SP stimulated the production of IL-1β, IL-6, and TNF-α*in vitro* in human dental pulp fibroblasts with severe orthodontic root resorption **(Rhee *et al*, 2009).**

1.4 Role of Cytokines and Growth Factors

1.4.1 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 4.

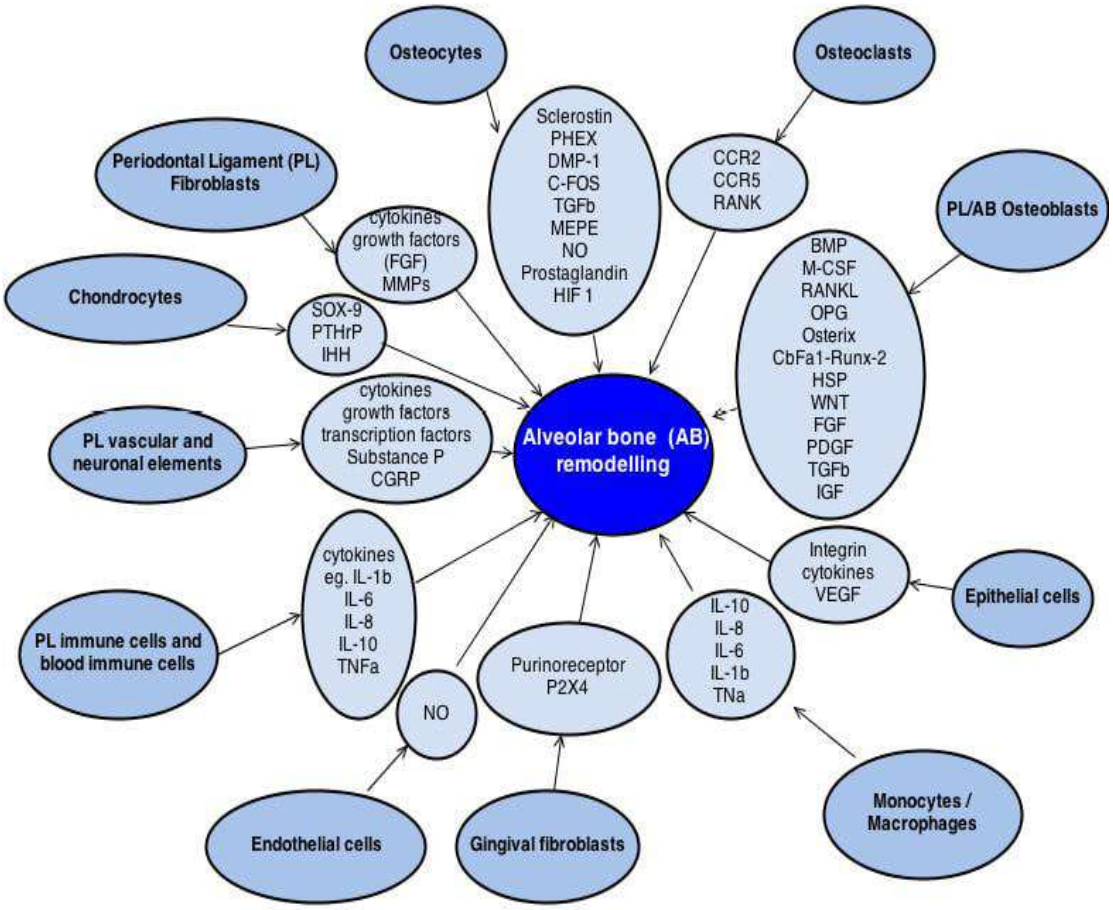

Figure 4 Cellular network in tooth remodeling **(Henneman S, *et al* 2008)**

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 **(Iwasaki *et al* 2001).**

These molecules modulate cell growth, proliferation, cell migration, differentiation, gene expression and cell specific functions.

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**(Megat Abdul Wahab *et al*,2008).**

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**(Yano S *et al* .,2005).**

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) **(Gluhak-Heinrich J, *et al*., 2006).**

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.

Integrins are also produced by osteoblasts. VEGF is produced by vascular endothelial cells, osteoblasts, osteoclasts and fibroblasts **(Gluhak-Heinrich J, *et al*., 2006).**

PHEX and DMP 1 regulate fibroblast growth factor.

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 **(Miyauchi A *et al.* 2006).**

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 **(Hoshi *K et al*. 2014).**

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 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**(You L, Temiyasathit S,2008)**

1.4.2 Role of growth factors

Bone morphogenetic proteins are a group of growth factors with cytokine properties . **(malone *et al* ,2007**)BMP 2 and BMP 7 are produced by osteoblasts and are involved in osteoblast differentiation.

BMP 2 also plays role in cementoblast differentiation.

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 **(Jacobs CR *et al*, 2010).**

1.4.3 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 **(Jacobs CR *et al*, 2010).**

1.4.4 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 **(Bonewald LF. (2006).**

1.5.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) **(Bonewald L. 2006).**

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 **(Ahuja SS*et al.* 2003).**

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.

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 which contains an Arg-Gly-Asp (RGD) motif that is known to promote osteoclast attachment through integrins & CD4 **(Ahuja SS *et al*. 2003).**

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 **(Hoshi K *et al.* 2014)**.

1.5.1Role of monocytes and macrophages

Activation of monocytes/macrophages produces several proand anti-inflammatory cytokines such as IL-1 beta, IL-6, Il-8, IL-10 TNF alpha**(Hoshi K, *et al*. 2014).**

1.5.2Role 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**(Gluhak-Heinrich J *et al*., 2006).**

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) **(Ahuja SS *et al*. 2003).**

1.5.3Role 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 **(Hoshi K *et al.* 2014).**Production of nitric oxide and prostaglandin E (2) by primary bone cells is shear stress dependent **(You L, *et al*. 2008)**

1.5.4.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 **(Bakker and soetima.,2001)**

1.5.5RANK 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) **(Klein-Nulend J *et al*.,1995)**

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**(Klein-Nulend J *et al*.,1995)**

1.5.6Regulation

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 **(E. Serra *et al*., 2003).**

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 **(Gluhak-Heinrich J *et al*., 2006).**

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. **(Hoshi K *et al.* 2014)**.

Integrins transmembrane receptors attach with other cells or ECM induces signaling pathways by changing intracellular Ca2+ regulate inositol lipid turn over & phosphorylation of intracellular proteins. MLO-Y4, a product of osteocyte stimulates surface lining osteoblasts. MAPK ERK ., MAPK JNK, MAPK p38 and MAPK ERK -5 induce cell differentiation and proliferation. **(Matsumoto T *et al*.,2013).**

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**(Burstone *et al.*, 1962).**

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 **(Matsumoto T *et al*.,2013).**

Chapter 2 Discussion

Based on the researches knowledge we shown in our research , it can be concluded that rate of tooth movement depends on bone remodeling which is a result of inflammatory process after orthodontic forces are applied on the teeth. The role of chemical mediators such as cytokines, interleukins, growth factors, RANKL receptors and osteoprotegerins in the processes of bone remodeling is considered when planning orthodontic tooth movement. Also, care should be taken when prescribing medications during orthodontic tooth movement because some medications like NSAIDs, Bisphosphonates, exogenous thyroxin, steroids, etc. can increase or decrease tooth movement.

There is progress in the development of biomarkers to better understand the ongoing biological processes involved with OTM. On the basis of sequential reactions and released substances, numerous substances have been proposed as biomarkers. Knowledge regarding stem cell development and osteoblastic and osteoclastic activity involved in bone formation and resorption, respectively,

the research on biology of tooth movement is on the right track and can accommodate new theories as well as better accelerating techniques.

Chapter 3 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.

suggestions

Many researches have proposed certain substances as biomarkers of OTM. There is progress in the development of biomarkers to better understand the ongoing biological processes involved with OTM.

On the basis of sequential reactions and released substances, numerous substances have been proposed as biomarkers. Knowledge regarding stem cell development and osteoblastic and osteoclastic activity involved in bone formation and resorption, respectively, can be useful in the identification of potential OTM biomarkers as well. There is also the question of how to obtain useful samples by ethical and noninvasive methods.

To this end, saliva and GCF are two common, noninvasive methods of collecting samples associated with OTM. The clinical use of these biomarkers is still an issue. Therefore, the determination of suitable OTM process biomarkers remains a challenging task. In this research, we proposed one potential marker for each phase during OTM, that is, ALP (bone formation), TRAP5a (bone resorption), LDH (inflammation), and DSPP (root resorption).

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