DIFFERENCES IN BONE TURNOVER MARKERS IN PRE AND POST-MENOPAUSAL WOMEN RECEIVING ORTHODONTIC TREATMENT

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Abstract

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Objective: The aim of our study was to investigate the differences in bone turnover marker levels in pre and post-menopausal women receiving orthodontic treatment. **Methods:** Twenty-eight orthodontic patients were recruited from CWRU and faculty members' private practices. Subjects were screened and excluded according to the selection criteria. Sixteen control subjects (mean age 33.2 ± 8.4 years) and 12 post-menopausal (PM) subjects (mean age 63.4 ± 8.1 years) participated and their gingival crevicular fluid (GCF) was taken from 4 sites; 2 from the anterior and 2 posterior teeth. Samples were taken at pre orthodontic activation (T_0) and 24 hours after activation (T_1). GCF samples are stored in -80c and RANKL, osteopontin and ostonection level were investigated by ELISA. **Results:** All biomarker levels in Control and PM groups had similar increase of biomarker concentration after activation without statistical significant. At T_0, RANKL in PM was significantly lower than in control group (Control 6.74pg/ml$\pm$ 5.45 vs PM 3.69 pg/ml$\pm$ 3.52, p-value=0.035*), SPARC in PM was significantly higher than in control (Control 325.10pg/ml$\pm$ 231.21 vs PM 872.8 pg/ml$\pm$ 409.92, p-value= 0.026*) and OPN had no significant difference. **Conclusions:** Findings in our study suggest that although there is a difference in baseline bone turnover markers in PMW versus controls, there is no difference in the initial change of these biomarkers in PMW vs. controls. This suggests that although underlying bone differences between these groups may exist in periodontally healthy subjects, orthodontic activation does not trigger an exaggerated response in PMW.

INTRODUCTION

According to the American Association of Orthodontics (AAO), number of adult patients increased 14 percent from 2010 to 2012, a record high of 1.2 million patients age 18 and older. According to the AAO's survey, 58 percent of the adult patients were female. Practices across the nation are experiencing higher number of female adult patients. Orthodontic tooth movement is based on bone remodeling. As post-menopausal women seek orthodontic treatment, their treatment time, outcome, oral health and sub sequential bone loss becomes a concern. Adult women face a major risk in bone loss and subsequent fracture as they enter menopause (Cheung et al. 2004 and Luo et al. 2014). At post menopause, bone remodeling rates double and triple 13 years later. They also remain elevated in osteoporosis. This change contributes to increases in age-related skeletal fragility in women (French et al. 2008). As estrogen level becomes deficient, micro-architectural deterioration of bone tissue and low bone mass can occur. Dual energy X-ray absorptiometry (DXA) is the most reliable way to determine bone mineral density (BMD), which is one of the major risk factors for fractures from osteoporosis (Miller et al. 1995). However, only a few BMD measurements are taken among patients in high-risk groups because of no symptoms appearing prior to fractures (Payne et al. 1999). A large proportion of postmenopausal women with a high risk of fractures from osteoporosis still remain undiagnosed (Yamashita et al. 2013). About 54 million Americans have osteoporosis

and low bone mass, placing them at increased risk for osteoporosis. Several researches also linked osteoporosis to be a risk factor for tooth loss and reduced mandibular bone mass and density (Lerner. 2006 and WHO. 1994). The World Health Organization (WHO) has established criteria for making the diagnosis of osteoporosis, as well as determining levels that predict higher chances of fractures. These criteria are based on comparing the bone mass density (BMD) of the patient with that of a typical healthy, young female's. BMD values that fall well below the average for the healthy, young females (stated statistically as 2.5 standard deviations below the average) are diagnosed as osteoporotic. Many women over 50 years of age still have not had adequate screening and testing for osteoporosis. Moreover, knowledge of changes in healthy, periodontitis free post-menopausal females are not fully understood when orthodontic forces are applied.

The aim of this study is to investigate the difference in bone turnover markers in gingival crevicular fluid between pre and post –menopausal women receiving orthodontic treatment.

Objective

1.) To determine the differences in Bone turnover biomarkers levels in the GCF in pre- and post- menopausal women receiving orthodontic treatment.
2.) To determine if difference in the changes in pre and post-menopausal women before and after activation of orthodontic treatment.

Hypotheses:

1.1 *Null*: There are no differences between Bone turnover markers levels in the GCF in pre- vs. post- menopausal woman undergoing orthodontic treatment.

1.2 *Alternate*: There are significant differences between bone turnover markers levels in the GCF in pre- vs. post- menopausal women undergoing orthodontic treatment.

2.1 *Null*: There are no differences in the change of Bone turnover markers levels before and after orthodontic activation in the GCF in pre- vs. post- menopausal woman undergoing orthodontic treatment.

2.2 *Alternate*: There are significant differences in the change of Bone turnover markers levels before and after orthodontic activation in the GCF in pre- vs. post- menopausal woman undergoing orthodontic treatment.

LITERATURE REVIEW

Post-Menopause and Osteoporosis

The average age of natural menopause in Western societies is estimated to be 51 years: climacteric women are at increased risk of chronic diseases such as osteoporosis and cardiovascular disease (Cheung et al. 2004). In 2005, a consensus from World Health Organization (WHO) defines post-menopausal stage as begins at the time of the Final Menstrual Period (FMP), regardless of whether the menopause was induced or spontaneous although it is not recognized until after 12 months of amenorrhea.

Postmenopausal osteoporosis (PMO) is osteoporosis that occurs after menopause because of the decrease in estrogen. The ovariectomized rat is a useful animal model for the study of osteoporotic bone related diseases caused by estrogen deficiency. Results from osteoporosis- induced animal models suggest a progressive loss of bone matrix through a process similar to what occurs during PMO (French et al. 2008 and Miller et al. 1995). In 2014, Luo et al. showed that ovariectomy promotes alveolar bone resorption in rats with experimental periodontitis and the possible underlying mechanism may be due to the decreased IL-10 and increased IL-6, OPG, and RANKL in ovariectomized periodontal tissues.

Osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissue. It is defined as a decrease in bone mass and strength leading to increased propensity to fracture (Payne et al. 2005). Osteoporosis

is clearly associated with skeletal bone loss and an increased incidence of vertebral, wrist and skeletal fractures (Payne et al. 1999). Estrogen deficiency is the dominant pathogenetic factor for osteoporosis in post-menopausal women. While the systemic effects of osteoporosis and estrogen deficiency have been known for years, the relationship between these two entities and alveolar bone loss has been examined only recently. Alveolar cancellous bone structure begins changing even in early postmenopausal women, and this structure varies in close relationship to bone turnover markers (Yamashita et al. 2013). Recent research focused on post-menopausal women with history or active periodontitis and its relationship to bone turnover and boner loss (Payne et al. 1999 and Lerner 2006). Diagnosing bone loss in any patient including post-menopausal women usually involves dual-energy X-ray absorptiometry (DXA) to evaluate bone mineral density loss in the lumbar vertebrae (WHO study group, 1994). However, for osteoporosis screening, quantitative ultrasound has gained more popularity recently as it is a non-invasive procedure (Jin et al. 2010). Bone quality is evaluated based on its micro-architechture, turnover, damage accumulation and mineralization of the tissue (Eastell and Blumsohn, 1997 and Singer et al. 2008). Bone turnover markers have been used in assessing risk of osteoporosis and fracture before actual problems appear (Garneo et al. 2000). Bone turn over markers can illustrate an ongoing bone metabolic activity, making it a tool to predict future changes in bone dynamic before actual bone loss. Deviations in bone turnover and consequent periodontal problems influence the response to orthodontic forces, and this should be taken into consideration when planning orthodontic

treatment in adult patients with metabolic bone disease, especially postmenopausal females or those on chronic medication affecting bone metabolism (Sidiropoulou et al. 2007).

Bone

Bone is a dynamic and continuously changing material that comprised of four major components: cells, extracellular matrix of collagen fibers, mucopolysacharide ground substance and calcium salts. Because remodeling is a continual state in normal bone, teeth are able to move and be moved throughout life.

-Bone formation

According to Gilbert (Gilbert, 2000) three distinct lineages generate the skeleton. The axial skeleton is generated by the somites, the lateral plate mesoderm generates the limb skeleton, and the branchial arch and craniofacial bones and cartilage are formed by the cranial neural crest. There are two major modes of bone formation, or osteogenesis, and both involve the transformation of a preexisting mesenchymal tissue into bone tissue. The direct conversion of mesenchymal tissue into bone is called **intramembranous ossification**. This process occurs primarily in the bones of the skull. In other cases, the mesenchymal cells differentiate into cartilage, and this cartilage is later replaced by bone. The process by which a cartilage intermediate is formed and replaced by bone cells is called **endochondral ossification**. Protective casing for the skulls is comprised of neurocranium bones and bones making up the face are known as viscerocranium. The membranous

neurocranium (neurocranium with intramembranous ossification) comprise of flat bone such as frontal bone, parietal bones, occipital bone, squamous part of temporal bones, nasal and lacrimal bones. The chondrocranium is formed by cartilaginous cells around the brain beginning at the notochord. The base of occipital bone, sphenoid and ethmoidal bones are formed from the hypophysial cartilage and the trabeculae cranii respectively. The lesser and greater wing of the sphenoid bone are also of endochondral origin. Viscerocranium also has both intramembranous and endochondral ossification. The maxilla, zygomatic bone, vomer, palatine bone, and mandible are of intramembranous ossification. The condyle of the mandible, however, has endochondral ossification along with the 3 ear bones; stapes, malleus and incus, and the styloid process.

-Alveolar bone structure

Alveolar process is an extension of the maxilla or the mandible that houses the dentition. The alveolar bone lies on the basal bone and its development is dependent on tooth eruption. When teeth are absent, the alveolar bone undergoes resorption and becomes involute. Haversian bone and compacted bone lamellae forms the external plate of the alveolus. The inner socket wall of thin, compact bone called the alveolar bone proper, which is seen as the lamina dura in radiographs (Carranza, 2014). Histologically, it contains a series of openings (cribriform plate) through which neurovascular bundles link the periodontal ligament with the central component of the alveolar bone, the cancellous bone. A compartment of spongy bone

or cancellous trabeculae lies between these two compact layers, which act as supporting alveolar bone. The interdental septum consists of cancellous supporting bone enclosed within a compact border. The coronal rim of the alveolar bone forms the alveolar crest, which generally parallels the cemento-enamel junction at a distance of 1-2 mm apical to it.

-Alveolar bone formation

Alveolar bone is formed during fetal growth by intramembranous ossification and consists of a calcified matrix with osteocytes enclosed within spaces called lacunae (Carranza, 2014). The osteocytes extend processes into canaliculi that radiate from the lacunae. Bone growth occurs by apposition of an organic matrix that is deposited by osteoblasts. Haversian systems (osteons) are the internal mechanisms that bring a vascular supply to bones too thick to be supplied only by surface vessels. These are found primarily in the outer cortical plates and the alveolar bone proper.

Bone remodeling

Remodeling is the replacement of old tissue by new bone tissue. The maintenance of bone is achieved by a fine balance between bone formation and bone resorption. Bone remodeling occurs through the coordinated action of osteoblasts and osteoclasts, involving the removal of mineralized bone by osteoclasts followed by the formation of bone matrix through the osteoblasts (Crockett et al. 2011). The activities of both types of cells are combined into defined anatomical spaces called basic

multicellular units (BMUs). A remodeling cycle begins with the activation of a new BMU on a previously inactive surface of bone. **Osteoblasts** are bone-forming cells of mesenchymal origin. They are connective tissue cells found at the surface of bone secreting bone matrix. When osteoblast becomes entrapped in the bone they are transformed into osteocytes or become bone lining cells. The mesenchymal stem cell that gives rise to osteoblasts can also produce cells of other lineages. It is also possible that osteoblast precursors can differentiate into or derive from adipocytes and marrow support cells (Dimitros et al. 2006). Osteoblasts can be buried as osteocytes, remain in the bone surface as lining cells, or undergo apoptosis. Differentiation of osteoblasts is regulated by the transcription factors bone morphogenetic proteins (BMPs), osteopontin (OPN) and Osterix (Cardoso et al. 2009 and Kim et al. 2012). Osteoblasts express at least two cytokines essential for osteoclast differentiation; they are receptor activator of NF-kappaB ligand (RANKL) and macrophage colony stimulating factor (M-CSF). They also produce type I collagen and is responsible for mineralizing the tissue (Aubin and Triffitt, 2002). The differentiation and activation of osteoclasts are tightly regulated by osteoblasts. **Osteoclasts** are bone-resorbing cells. They are large, multinucleate cells that form through the fusion of precursor cells. Unlike osteoblasts, which are related to fibroblasts and other connective tissue cells, osteoclasts are formed by the attraction of hemopoietic myelomonocytic precursors. Osteoclast differentiation initially depends on signaling through c-fms, the receptor for macrophage colony stimulating factor (MCSF), in mononuclear precursor cells, which up regulates expression of

RANK (Crockett et al., 2011). Its ligand, RANKL, is expressed in osteoblasts and stromal cells in response to PTH and stimulation by the active dihydroxy form of vitamin D_3 (1, 25 Vitamin D_3) (Leibbrandt and Penninger, 2008). Osteoblasts initiate fusion and subsequent attachment of multinucleated cells to the bone surface. Activation of osteoclasts are also regulate by osteoprotegerin (OPG) produced by osteoblasts. As RANKL adhere to its receptor, RANK ion the osteoclast cell surface to stimulate its activity, OPG intercept the conjugation by attaching the RANKL. If RANKL is coupled with OPG, it will not be able to connect to its receptor, RANK, to stimulate osteoclasts. The most characteristic feature of a mature osteoclast is the presence of ruffled boarders and clear zone (Vaananen and Zhao. 2002). Osteoclasts resorb bone matrix and its materials until activation from osteoblasts decreases. The resorption phase is terminated, possibly by osteoclast apoptosis from lack of osteoblastic stimulation, and after a brief reversal phase, a group of osteoblast is recruited to fills in the resorption cavity with new bone. The net result is the replacement of a packet of old bone with new bone.

Summary of bone remodeling process involves the coupling of bone formation and bone resorption and consists of five phases:

1. Activation: preosteoclasts are stimulated and differentiate under the influence of cytokines and growth factors into mature active osteoclasts
2. Resorption: osteoclasts digest mineral matrix (old bone)
3. Reversal: end of resorption

4. Formation: osteoblasts synthesize new bone matrix

5. Quiescence: osteoblasts become resting bone lining cells on the newly formed bone surface

-Systemic influence on bone remodeling

Hormones are possibly the most crucial modulators of bone formation (Weitzmann et al. 2006). Parathyroid hormone (PTH) regulates serum calcium concentration. It is a potent stimulator of bone resorption and has biphasic effects on bone formation (Lips. 2006). PTH acts on osteoblast up regulating RANKL and, consequently, stimulates osteoclasts maturation and activity. PTH inhibits collagen synthesis with high concentrations of PTH, but prolonged intermittent administration of this hormone produces increased bone formation (Dempster et al. 1993). Plasma PTH tends to increase with age, and this may produce an increase in bone turnover and a loss of bone mass, particularly of cortical bone. Other systemic hormones are important in regulating skeletal growth. Growth hormone, acting through both systemic and local insulin-like growth factor (IGF) production, can stimulate bone formation and resorption (Rosen and Donahue. 1998). Glucocorticoids are necessary for bone cell differentiation during development, but their greatest postnatal effect is to inhibit bone formation (Advani et al. 1997). This is the major pathogenesis in glucocorticoid-induced osteoporosis. Indirect effects of glucocorticoids on calcium absorption and sex hormone production may, however, increase bone resorption.

Thyroid hormones can also stimulate bone resorption and formation and are critical for maintenance of normal bone remodeling.

The most important systemic hormone in maintaining normal bone turnover is estrogen (Pacifici. 1998 and Weitzmann and Pacifici. 2006). Reduction of Estrogen can lead to an increase in bone remodeling where resorption rate out speed formation rate and bone mass decreases. This can be observed in postmenopausal women. Estrogen mediated through one specific type of cell surface receptor called the estrogen receptor alpha (ERα), which binds and transports the hormone into the nucleus of the cell where the receptor-hormone complex activating specific genes (Zallone et al. 2006). ERα receptors are found on the surface of osteoblasts, as is estrogen receptor-related receptor alpha (ERRα), which may play an auxiliary role in regulating bone cells (Bonnelye and Aubin. 2005). Alternatively, estrogen may also inhibit local factors that impair bone formation or enhance local factors that stimulate bone formation.

-Local factor influencing bone remodeling

In an inflammatory process, there are several cells and mechanisms which can theoretically enhance RANKL. Interleukin 1-Beta (IL-1B) (Dewhirst et al. 1985), tumor necrosis factor-alpha (TNF-a) (Bertolini et al. 1986) and interleukin-6 (IL-6) (Ishimi et al. 1990) were able to stimulate bone resorption by up-regulating RANKL. IL-1, TNF-a, and IL-6 have been found to be abundantly expressed in inflamed gingiva, and increased levels have been shown in the crevicular fluid from patients

with periodontitis (Mogi et al. 1999 and Graves and Cochran. 2003). Thus, the same types of cytokines have been implicated as stimulators of bone resorption in periodontitis, rheumatoid arthritis, and postmenopausal osteoporosis (Lerner. 2006). The group of bone resorbing cytokines currently includes IL-1, IL-6, IL-11, IL-17, TNF-a. These cytokines react directly to osteoblasts and has been shown that TNF-a can stimulate osteoclast formation in the absence of stromal cells/osteoblasts if a certain level of RANKL is present (Lam et al. 2000 and Wei et al. 2005). Other cytokines such as IL-4, IL-13, IL-18, IFN, and OPG have been shown to prevent bone loss by blockage of RANKL or reducing the productivity of RANKL from osteoblasts (Lawrence and Raisz.1999). Prostaglandins (PGE_2), a lipid that is formed in various bone cells from a precursor called arachidonic acid, maybe required for exercised-induced bone formation. PGE_2 synthesis is carried out by an enzyme called cyclooxygenase $_2$ (COX_2) and inhibitors of this enzyme can prevent bone formation in response to mechanical stress in animals. Other local factors include growth factors, nitrous-oxide and anti-inflammatory proteins.

RANKL

A receptor activator of NFkB (RANK) is present on the cell surface of osteoclasts precursor cells. When stimulated by PTH, osteoblasts up-regulate expression of RANK Ligand (RANKL), which binds to RANK, activating signaling pathways that promote osteoclast differentiation and survival (Yasuda et al. 1998 and Lacey et al. 1998). RANKL is produced by osteoblasts and is one of many signaling

molecules that facilitate cross-talk between the osteoblasts and osteoclasts and help coordinate bone remodeling (Theil et al. 2002). Osteoprotegerin (OPG), also released by osteoblasts, can also bind to RANKL, acting as a competitive substrate to prevent RANK and RANKL from coming in contact. The balance of RANKL/OPG may be crucial in determining bone turnover. In fact, animal studies showed that increased production of OPG leads to an increase in bone mass, while loss of the protein leads to osteoporosis and increased fractures (Takayanagi et al. 2005). Inhibitors of RANKL have also shown as potential treatment for osteoporosis in humans.

Osteopontin

Osteopontin (OPN) is a major non-collagenous bone matrix protein; it contains a motif of glycine-arginine-glycine-aspartic acid-serine (GRGDS). This 314 amino acid sequence promotes call attachment via cellular $\alpha_v\beta_3$ integrin. OPN have been suggested to enhance or regulate the adhesion, attachment, and spreading of osteoclasts to the bone surface during resorption. OPN is produced by osteoblasts and osteoclasts and plays a vital role in bone remodeling (Takano et al. 1994). OPN expressed in osteocytes and osteoblastic cells due to mechanical stress might induce migration of osteoclastic precursor cells to the bone surface and promote bone resorption. These results indicate that these cells responded to mechanical stress loaded on the bone with expression of the osteopontin gene. Following the increased expression of OPN in these cells, a 17-fold greater number of osteoclasts compared with the control and numerous resorption pits were observed on the pressure side of

the alveolar bone. OPN is an important factor triggering bone remodeling caused by mechanical stress. OPN participates in the attachment of osteoclasts to bone surface during bone resorption, bind to calcium and hydroxyapatite and bind to osteoclast vitronectin receptor. OPN is expressed temporarily in the stage of resorption and has been suggested to be involved in woven bone forming. Several studies showed OPN increasing after 12 hours of applying mechanical force (Terai et al. 1999). OPN is possibly a multifunctional protein that acts as a coupling factor for bone resorption and formation in the process of bone remodeling. Many recent researches suggest that RANKL and OPN concentration show significant increase after 24 hour of orthodontic activation, although it is possible that this is not the peak concentration (Hart. 2013 and Faulkner. 2012)

Osteonectin (SPARC)

Osteonectin or secreted protein acidic and rich in cysteine (SPARC) is the most abundant non-collagenous extracellular matrix protein in bone (Delany and Hankenson. 2009). Analysis of the skeletal phenotype of SPARC-null mice has provided excellent information on the function of this protein in bone. SPARC-null mice develop profound low-turnover that becomes progressively worse with age (Boskey et al. 2003); they also found decreased numbers of osteoblasts and osteoclasts, and a markedly decreased bone-formation rate. In the skeleton, osteonectin promotes osteoblast commitment, suppresses adipogenesis, and regulates

the balance between bone formation and resorption in response to PTH treatment. It is highly expressed early in osteoblastic differentiation, but its expression decreases as the cells acquire characteristics of mature osteoblasts. SPARC-null mice have decreased trabecular bone volume due to decreased trabecular number. Boskey et al concluded that bone resorption rate becomes higher than bone forming and result in reduced bone mass due to lack of osteonectin.

Osteoporosis and bone remodeling

The most common metabolic disorder is primary osteoporosis (Raisz. 1997). Studies have suggested that estrogen deficiency is important for the pathogenesis of osteoporosis in both men and women (Riggs at al. 1998). At menopause there is a transient, accelerated phase of bone loss that is followed by slower, sustained bone loss (Riggs et al. 2002). Although in men there is no abrupt cessation of gonadal function in the sixth decade of life, they do experience an age-related decrease in unbound sex steroids resulting from progressive increases in circulating sex hormone–binding globulin. In both men and women there is a steady decline in unbound (bioavailable) estrogen levels with aging, exacerbated in women at menopause by a marked decrease in estrogen levels. Studies using bone markers suggest that there is accelerated bone remodeling at menopause and that bone formation may increase overall, but that the rate is inadequate to replace the bone lost by resorption (Prestwood et al. 1995 and Eastell. 1998).

Orthodontic tooth movement

Orthodontic tooth movement is defined as mechanical stimulation causing remodeling of the periodontal ligament (PDL) and alveolar bone. Mechanical force is a key regulator of bone remodeling and of bone architecture in general (Jacobs et al. 2010). Early signals produced in response to mechanical stimuli include nitric oxide, prostaglandins and Wnt signaling proteins (Newald and Johnson. 2008). A precondition for these remodeling activities and tooth displacement are the occurrence of an inflammatory process. Vascular and cellular changes were the first events to be recognized and described, and a number of inflammatory mediators, growth factors, and neuropeptides have been demonstrated in periodontal supporting tissues. Orthodontic forces can cause mechanical injury to the PDL and cause alveolar bone resorption, although this damage can be resolved with remodeling of the PDL and alveolar bone (Mabuchi et al. 2002). Orthodontic tooth movement results from the resorption and formation of alveolar bone and the degradation and remodeling of the PDL. Osteoclastogenesis and dynamic changes to the collagenous composition of the PDL are key processes in initiating bone remodeling (Xin et al. 2010). In 1973, 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 (Storey et al. 1973). Later research has led to the hypothesis that inflammatory mediators are released following mechanical stimulus, triggering the biologic processes associated with alveolar bone resorption and apposition (Davidovitch et al. 1988). Among the local biochemical mediators are cytokines secreted by mono- nuclear cells and leukocytes. Cytokines can provoke the

synthesis and secretion of numerous substances that for the molecular basis for cell-to-cell communication, including prostaglandins (PGs) and growth factors, thus interacting directly or indirectly with bone cells60. Two theories are currently used in describing orthodontic tooth movement.

1. Biomechanical orthodontic tooth movement

Mechanical distortion to cell membrane is the key activator in this theory. As this is a form of sterile insult to the periodontal tissue, remodeling could be perceived as an attempt to remove ischemic bone under the hyalinized tissue (Melsen 2001). This produces prostaglandins which feed back onto the cell membrane binding to receptors which then stimulate second messengers and elicit a cell response. At the site where mechanical insult is present, cells may communicate with each other by means of cytokines interactions. Finally, bone is laid down in the tension site and resorbs at the pressure site. It is not clear how tissues discriminate between tension and pressure. In 2004, Roberts-Harry and Sandy found that cells which are rounded up show catabolic changes whereas flattened cells have anabolic effects.

2. Bone bending, piezoelectric and magnetic forces

During the 1960s, there was considerable interest in piezoelectricity as a stimulus for bone remodeling. This theory initiated because it was noted that distortion of crystalline structures generated small electrical charges, which potentially may have been responsible for signaling bone changes associated with mechanical forces.

Three stages are characterized after moderate and continuous force is applied to a tooth. The initial strain is seen between 4 to 7 days after force is initiated. The lag phase is then followed by the bending and progressive tooth movement is the final stage (Graber. 2005). The rate limiting step in the final stage is known as frontal resorption, which determines the speed of orthodontic correction. The strain on the extra cellular matrix causes fluid displacement in alveolar bone canaliculi. Osteocytes and osteoblast activation occurred due to the stimulation of PDL fibroblasts after fluid displacement. The periodontal ligament (PDL) neurons secrete neuro- peptides such as substance P, calcitonin gene related peptide (CGRP) when nociceptor pain is present by mechanical force. These peptides along with prostaglandin E-2 (PGE-2) and other cytokines can dilate PDL capillaries; this can lead to the release of immune competent cells. Endothelial cells and osteoblasts secrete essential mediator for bone angiogenesis and in bone formation call vascular endothelial growth factor (VEGF). The ECM remodeling is followed by cytoskeletal re-organization in osteoblastic cells. Cytoskeletal re-organization leads to phosphorylation of cellular proteins including extracellular signal-regulated kinases, which triggers signal transduction via integrins/ fibronectin/kinase- pathway (Eduardo et al. 2005). 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 (Gyda et al. 2001). Macrophage colony-stimulating factor (M-cfs) is a secretory product of osteoblasts, which work with other cytokines to regulate the differentiation of osteoclast precursors to mature osteoclasts

(Takatsuka et al. 1998). M-cfs with OPG ligand can also enhances osteoclastogenesis. Recent works from University of Hong Kong 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 (IHH) are very significant in understanding the molecular biology of orthodontic tooth movement force transduction (Huang et al. 2001). Indian Hedgehog (IHH) is protein involved in chondrocyte differentiation, proliferation and maturation especially during endochondral ossification by effecting cartilaginous differentiation through negative loop to control PTHrP. PTHrP regulates extracellular matrix gene expression in cementoblasts and inhibits cementobalst-mediated mineralization in vitro (Vortkamp er at. 1996), all mechanisms of interest and importance in orthopedic growth modification. Huang et al also found that SOX 9 directly regulates the Type II collagen gene. 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.

The strain and stretch effects caused by orthodontic forces induce PDL fibroblasts, osteocytes, osteoblasts and osteoclasts lead to the production of a number of messenger molecules. Periodontal ligament and its immune cells produce pro-inflammatory cytokines (IL-1 beta, Il-6, TNF alpha) to modulate cell growth, proliferation, cell migration, differentiation, gene expression and cell specific functions (McLean. 2009). IL-1β is considered an important cytokine in tooth movement due to its pleotropic effects. This cytokine plays a key role in immune

regulation and inflammatory processes by inducing expression of many effector proteins. It is a modulator of bone resorption in inflammatory settings byt inducing RANKL expression and synergizing RANKL to promote osteoclast differentiation. (Lee et al. 2010). Tumor 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. The orthodontic tooth movement activates osteoblasts. In response, osteoblasts produce 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-β), 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. Osteoblast differentiation is also induced by hedgehogs (IHH) and core binding transcription factor alpha-1 (cbfa1). Ischemia and hypoxia occur on the pressure side as a result of reduced blood supply from orthodontic forces, can cause HIF-1to inhibits Wnt signaling in osteoblasts, which inhibiting osteoblast differentiation as Wnt signaling is responsible for osteoblast differentiation. Epithelial cells produce integrins,

cytokines, vascular endothelial growth factor (VEGF). Integrin is produced by osteoblasts and play an important role in cell signaling mechanism.. VEGF is produced by vascular endothelial cells, osteoblasts, osteoclasts and fibro- blasts. 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 number and distribution patterns of RANKL- and RANK-expressing osteoclasts change when excessive orthodontic force was applied to periodontal tissue, and IL-1b and TNF-a were expressed in osteoclasts in pathologic status rat periodontal tissues. Shiotani et al. have also shown the presence of RANKL in periodontal tissues during experimental tooth movement of rat molars. Therefore, it is suggested that in response to mechanical stress, RANKL is regulated by inflammatory cytokines in the PDL. The number and distribution patterns of RANKL and RANK-expressing osteoclasts change when excessive orthodontic force is applied to periodontal tissues. Aihara et al. and Kim et al. showed

the presence of RANKL in periodontal tissues during experimental tooth movement of rat molars. Kanzaki et al. demonstrated that transfer of the RANKL gene to the periodontal tissue activated osteoclastogenesis and accelerated the amount of experimental tooth movement in rats. In contrast, OPG gene transfer inhibited RANKL-mediated osteoclastogenesis and inhibited experimental tooth movement. Therefore, it is suggested that PGE2, inflammatory cytokines, and the RANKL–RANK system may be strong regulators of orthodontic tooth movement.

Gingival crevicular fluid study during orthodontic tooth movement

Gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate found in the gingival sulcus, where it tends to increase in volume with inflammation and greater capillary permeability. Serum is the primary source of the aqueous component of GCF; however, the gingival tissue through which the fluid passes, along with bacteria present in the tissue and gingival crevice, can modify its composition (Cimasoni 1983). With constituents of GCF being derived from a variety of sources, including microbial dental plaque, host inflammatory cells, host tissue, and serum, GCF varies according to the condition of the periodontal tissues. In addition to the cells, immunoglobulins, microorganisms, toxins, and lysosomal enzymes detected in GCF, the mechanism of bone resorption may also be related to the release of inflammatory mediators present in GCF. Recently, a number of GCF constituents have been shown to be diagnostic markers of active tissue destruction in periodontal diseases (Lamster and Novak 1992), although only a few studies have

focused on those involved in bone remodeling during orthodontic tooth movement. Mogi et al. found that GCF concentrations of IL-1b and IL-6 were significantly higher in a group with severe periodontal disease compared with controls, and Yavuzyilmaz et al. demonstrated the GCF IL-1b and TNF-a levels had a positive correlation to mean pocket depths, suggesting that the cytokines may be involved in the pathogenesis of periodontal diseases. An increased concentration of RANKL and decreased concentration of OPG were detected in GCF from patients with periodontitis, while the ratio of RANKL concentration to that of OPG in GCF samples was significantly higher for patients with periodontal disease than for healthy subjects. Taken together, these data suggest that RANKL and OPG contribute to osteoclastic bone destruction in periodontal disease. Nishijima et al.[53]found an increased concentration of RANKL in GCF during orthodontic tooth movement, and the ratio of concentration of RANKL to that of OPG in the GCF was significantly higher than in control sites in another study. Further, Kawasakiet al. reported that the age-related decrease in amount of tooth movement maybe related to a decrease in RANKL⁄OPG ratio in GCF during the early stages of orthodontic tooth movement. Consequently, analysis of GCF samples may provide a better understanding of the biochemical processes associated with tooth movement, potentially helping clinicians make therapeutic choices based on qualitative and quantitative information.

REFERENCES

1. Advani S, Lafrancis D, Bogdanovic E, Taxel P, Raisz Lg, Kream Be. Dexamethasone Suppresses In Vivo Levels Of Bone Collagen Synthesis In Neonatal Mice. Bone 1997; 20:41-46.
2. Aihara N, Otsuka A, Yamaguchi M, Okada H, Utunomiya T, Yamamoto H Et Al. Localization Of Rankl And Cathepsin K, B, And L In Rat Periodontal Tissues During Experimental Tooth Movement. Orthod Waves 2005;64:107
3. Aubin JE, Triffitt JT. Mesenchymal stem cells and osteoblast differentiation. In: Bilezikian JP, Raisz LG, Rodan GA, eds. Principles of Bone Biology, 2nd edn. Academic Press: San Diego, 2002. pp. 59–81
4. Bertolini Dr, Nedwin Ge, Bringman Ts, Smith Dd, Mundy Gr. Stimulation Of Bone Resorption And Inhibition Of Bone Formation In Vitro By Human Tumour Necrosis Factors. Nature. 1986. 319:516-518
5. Bonnelye, E., and Aubin, J.E. Estrogen Receptor-Related Receptor Alpha: A Mediator Of Estrogen Response In Bone. J. Clin. Endocrinol. Metab. 2005. 90:3115–3121
6. Boskey Al, Moore D, Amiling M, Canalis E, Delany Am. Infrared Analysis Of The Mineral And Matrix In Bones Of Osteonectin-Null Mice And Their Wildtype Controls. J Of Bone and Min Res. 2003. Vol 18, No. 6.
7. Cardoso, L, Herman, BC, Verborgt, O, Laudier, D, Majeska, R J and Schaffler, MB. Osteocyte Apoptosis Controls Activation of Intracortical Resorption in Response to Bone Fatigue. J. Bone Miner. Res. 2009. 24, 597-605.

8. Carranza Fa, Newman Mg, Takei Hh, Klolevld Pr. Carranza's Clinical Periodontology , 12th Edition Published By Elsevier. 2014
9. Cheung A, Chaudhry R, Kapral M, Jackevicius C and Robinson G. Bmc Womens Health. 2004; 4(Suppl 1): S23
10. Cimasoni G. Crevicular fluid updated. In: Myers HM, editor. Monographs in Oral Sciences. Basel: Karger; 1983. pp. 1–152
11. Crockett J, Rogers MJ, Coxon FP, Hocking LJ and Helfrich M. Bone Remodeling At A glance. April 1, 2011 J Cell Sci 124, 991-998.
12. Delany Am, Hankenson Kd. Thrombospondin-2 And Sparc/Osteonectin Are Critical Regulators Of Bone Remodeling. J Cell Commun Signal. 2009 Dec;3(3-4):227-38.
13. Davidovitch Z, Nicolay Of, Ngan Pw, Shanfeld Jl. Neurotrans-Mitters, Cytokines, And The Control Of Alveolar Bone Remodeling In Orthodontics. Dent Clin North Am 1988;32:411–35
14. Dempster Dw, Cosman F, Parisien M, Shen V. Anabolic Actions Of Parathyroid Hormone On Bone. Endocr Rev 1993;14:690-709
15. Dewhirst FE, Stashenko PP, Mole JE, Tsurumachi T. Purification and Partial Sequence Of Human Osteoclast-Activating Factor: Identity With Interleukin 1 Beta. J Immunol 1985. 135:2562-2568.
16. Dimitrios J. Hadjidakis and Ioannis I. Androulakis. Bone Remodeling. Ann. N.Y. Acad. Sci. 2006.1092: 385–396.

17. Eastell R, Blumsohn A. The Value Of Biochemical Markers Of Bone Turnover In Osteoporosis. J Rheumatol 1997;24:1215-7.
18. Eastell R. Treatment Of Postmenopausal Osteoporosis. N Engl J Med 1998;338:736-*746.*
19. D'Apuzzo F, Cappabianca S, Ciavarella D, Monsurrò A, Silvestrini-Biavati A, and Perillo L, "Biomarkers of Periodontal Tissue Remodeling during Orthodontic Tooth Movement in Mice and Men: Overview and Clinical Relevance," The Scientific World Journal, vol. 2013, Article ID 105873, 8 pages, 2013
20. Faulkner Mg. Gingival Crevicular Fluid (Gcf) Levels Of Interleukin-6 (Il-6), Sgp 130 And Sil-6r During Orthodontic Tooth Movement. Master's Thesis. Unlv 2011.
21. French DL, Muir JM, Webber C, The Ovariectomized, Mature Rat Model Of Postmenopausal Osteoporosis: An Assessment Of The Bone Sparing Effects Of Curcumin, Phytomedicine, Vol. 15, No. 12, Pp. 1069–1078, 2008.
22. Garnero P, Sornay-Rendu E, Claustrat B, Delmas Pd. Biochemical Markers of Bone Turnover, Endogenous Hormones and The Risk Of Fractures In Postmenopausal Women: The Ofely Study. J Bone Miner Res 2000;15:1526-36
23. Graber Tm, Vanarsdall Rl, Vih Kwl. Orthodontics, Current Principles and Techniques, 4th Edition. St. Louis. Mosby. 2005

24. Gilbert SF. Developmental Biology. 6th edition. Sunderland (MA): Sinauer Associates; Osteogenesis: The Development of Bones. 2000.
25. Graves D, Cochran D. The Contribution Of Interleukin-1 And Tumor Necrosis Factor To Periodontal Tissue Destruction. J Periodontol 2003. 74:391-401
26. Gyeda M, Corisdeo S, Zaidi M, Troen B. Macropgae Colony-Stimulating Factor Suppresses Osteoblast Formation. Bioc and Biop Res Comm. Vol 285; 2, 13 July 2001, Pages 328–334
27. Hart a. Rankl and Opg Levels In Response To Orthodontic Forces. Master's Thesis. Uni Of Tenn. 2012.
28. Huang W, Chung UI, Kronenberg HM, de Crombrugghe B. The chondrogenic transcription factor Sox9 is a target of signaling by the parathyroid hormone-related peptide in the growth plate of endochondral bones. Proc Natl Acad Sci U S A. 2001 Jan 2;98(1):160-5
29. Ishimi Y, Miyaura C, Jin Ch, Akatsu T, Abe E, Nakamura Y. Il-6 Is Produced By Osteoblasts and Induces Bone Resorption. J Immunol. 1990. 145:3297-3303
30. Jacobs CR, Temiyasathit S, Castillo AB. Osteocyte Mechanobiology and Pericellular Mechanics. Annu. Rev. Biomed. Eng. 2010. 12, 369-400.
31. Jin N, Lin S, Zhang Y, Chen F. Assess The Discrimination Of Achilles Insight Calcaneus Quantitative Ultrasound Device For Osteoporosis In Chinese

Women: Compared With Dual Energy X-Ray Absorptiometry Measurements. Eur J Radiol 2010;76: 265-8

32. Katagiri T. and Takahashi N. Regulatory mechanisms of osteoblast and osteoclast differentiation. Oral Diseases, 2002. 8: 147–159.
33. Kawasaki K, Takahashi T, Yamaguchi M, Kasai K. Effects of aging on RANKL and OPG levels in gingival crevicular fluid during orthodontic tooth movement. Orthod Craniofac Res. 2006;9:137–42.
34. Kim JY, Kim B, Jue S, Park JH, Shin J. Localization of osteopontin and osterix in periodontal tissue during orthodontic tooth movement in rats. The Angle Orthodontist: January 2012, Vol. 82, No. 1, pp. 107-114
35. Kim T, Handa A, Iida J, Yoshida S. Rankl Expression In Rat Periodontal Ligament Subjected To A Continuous Orthodontic Force. Arch Oral Biol 2007;52:244–50
36. Lacey D, Timms E, Tan H, Kelley M, Dunstan C, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian Y, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle W. Osteoprotegerin Ligand Is A Cytokine That Regulates Osteoclast Differentiation And Activation. Cell 1998; 93:165–176
37. Lacroix A, Jackson R, Aragaki A, Kooperberg C, Cauley Ja, Chen Z, Leboff M, Duggan D, Wactawski-Wende. J Opg And Srankl Serum Levels And Incident Hip Fracture In Postmenopausal Caucasian Women In The

Women's Health Initiative Observational Study. Bone. 2013 Oct;56(2):474-81.

38. Lam J, Takeshita S, Barker Je, Kanagawa O, Ross Fp, Teitelbaum Sl (2000). Tnf-Alpha Induces Osteoclastogenesis By Direct Stimulation Of Macrophages Exposed To Permissive Levels Of Rank Ligand. J Clin Invest 106:1481-1488
39. Lamster IB, Novak MJ. Host mediators in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. Crit Rev Oral Biol Med 1992;3:31–60.
40. Raisz L. Physiology and Pathophysiology Of Bone Remodeling. Clinical Chemistry August 1999 Vol. 45 No. 8 1353-1358
41. Lerner UH. Inflammation-Induced Bone Remodeling In Periodontal Disease And The Influence Of Post-Menopausal Osteoporosis. J Dent Res. 2006 Jul;85(7):596-607
42. Lee B, Kim TH, Jun JB, Yoo DH, Woo JH, Choi SJ, Lee YH, Song GG, Sohn J, Park-Min KH, Ivashkiv LB, Ji JD. Direct inhibition of human RANK+ osteoclast precursors identifies a homeostatic function of IL-1beta. J Immunol. 2010 Nov 15;185(10):5926-34.
43. Lindsay R. Prevention and Treatment of Osteoporosis. Lancet. 1993;341:801-805.
44. Lips P. Vitamin D Physiology. Prog Biophys Mol Biol. 2006;92(1):4-89
45. Liu Jm, Zhao Hy, Ning G, Zhao Yj, Chen Y, Zhang Zh, Sun Lh, Xu My, Chen Jl. Relationships Between The Changes Of Serum Levels Of Opg And Rankl

With Age, Menopause, Bone Biochemical Markers And Bone Mineral Density In Chinese Women Aged 20-75. Calcif Tissue Int. 2005 Jan;76(1):1-6.

46. Luo K, Souzhi M, Jianbin G, Yongling H, Fuhua Y and Yin X. Association between Postmenopausal Osteoporosis and Experimental Periodontitis. Biomed Research International. Volume 2014 (2014), Article Id 316134
47. Mabuchi R, Matsuzaka K, Shimono M: Cell Proliferation and Cell Death In Periodontal Ligaments During Orthodontic Tooth Movement. J Periodontal Res 2002;37:118–124
48. Masella R, Meister M. Current Concepts In The Biology Of Orthodontic Tooth Movement. Am J Of Orthod And Dentofacial Orthop. 2005. Volume 129, Number 4.
49. Matsuo K, Irie N. Osteoclast-osteoblast communication. Arch Biochem Biophys. 2008 May 15;473(2):201-9.
50. McLean RR. Proinflammatory cytokines and osteoporosis. Current Osteoporosis Reports.December 2009, Volume 7, Issue 4, pp 134-139
51. Melsen B. Tissue reaction to orthodontic tooth movement- a new paradigm. Eur J Orthod. 2001; 23. 671-681
52. Miller SC, Bowman BM, Jee WS, Available Animal Models Of Osteopenia—Small And Large. Bone, Vol. 17, No. 4, Pp. 117–123, 1995.
53. Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, Kojima K. Interleukin 1 Beta, Interleukin 6, Beta 2-Microglobulin, and Transforming Growth Factor-

Alpha In Gingival Crevicular Fluid From Human Periodontal Disease. Arch Oral Biol 1999. 44:535-539

54. Nayak BN, Galil K, Wiltshire W, Lekic PC. Molecular Biology Of Orthodontic Tooth Movement. J Dent Oral Health 2013 Vol 1: 101
55. Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K. Levels Of Rankl And Opg In Gingival Crevicular Fluid During Orthodontic Tooth Movement And Effect Of Compression Force On Release From Periodontal Ligament Cells In Vitro. Orthod Craniofac Res. 2006. May; 9(2):63-70
56. Pacifici R. Cytokines, Estrogen and Postmenopausal Osteoporosis—the Second Decade. Endocrinology 1998;139:2659-2661
57. Payne JB, Reinhardt R, Nummikoski P, Patil KD. Longitudinal Alveolar Bone Loss In Postmenopausal Osteoporotic/Osteopenic Women. Osteoporos Int. 1999;10(1):34-40.
58. Pilbeam, CC, Harrison, JR, Raisz, LG. Prostaglandins and Bone Metabolism. In Principles of Bone Biology. Academic Press. San Diego, California, USA. 2002. 979–994
59. Prestwood K, Pilbeam C, Raisz LG. Treatment of Osteoporosis. Annu Rev Med 1995;46:249-256.
60. Raisz LG. The Osteoporosis Revolution. Ann Intern Med 1997;126:458-462.
61. Riggs BL, Khosla S, Melton LJ. A Unitary Model for Involutional Osteoporosis: Estrogen Deficiency Causes both Type I and Type II

Osteoporosis in Postmenopausal Women and Contributes To Bone Loss In Aging Men. J Bone Miner Res 1998;13:763-773.

62. Riggs BL, Khosla S, Melton LJ 3rd. Endocr Rev. Sex Steroids and the Construction and Conservation Of The Adult Skeleton. 2002 Jun;23(3):279-302.
63. Roberts-Harry D and Sandy J. Orthodontics. Part 11: Orthodontic tooth movement. Brit Dent J 2004; 196, 391-394
64. Rosen CJ, Donahue LR. Insulin-Like Growth Factors and Bone—The Osteoporosis *Connection Revisited.* Proc Soc Exp Biol Med 1998;219:1-7.
65. Shiotani A, Shibasaki Y, Sasaki T. Localization Of Receptor Acti-Vator Of Nf-Kappab Ligand, Rankl, In Periodontal Tissues During Experimental Movement Of Rat Molars.J Electron Microsc 2001;50:365–9
66. Sidiropoulou-Chatzigiannis S, Kourtidou M, Tsalikis L. The Effect Of Osteoporosis On Periodontal Status, Alveolar Bone And Orthodontic Tooth Movement. A Literature Review. J Int Acad Periodontol. 2007 Jul;9(3):77-84.
67. Singer FR, Eyre D. Using Biochemical Markers of Bone Turnover In Clinical Practice. Cleve Clin J Med 2008;75:739-50
68. Storey E. The Nature of Tooth Movement. Am J Orthod 1973;63:292–314
69. Takano-Yamamoto T, Takemura T, Kitamura Y And Nomura S. Site-Specific Expression Of Mrnas For Osteonectin And Osteopontin Revealed By In Situ Hybridization In Rat Periodontal Ligament During Physiological Tooth Movement. J Histochem Cytochem. 1994, 42:885.

70. Takayanagi H. Mechanistic Insight into Osteoclast Differentiation in Osteoimmunology. J Mol Med. 2005;83(3):170-9

71. Takatsuka H, Umezu H, Hasegawa G, Usuda H, Ebe Y, Naito M, Shultz LD Bone remodeling and macrophage differentiation in osteopetrosis (op) mutant mice defective in the production of macrophage colony-stimulating factor. J Submicrosc Cytol Pathol. 1998 Apr;30(2):239-47

72. Terai K, Takeno-Yamamoto T, Ohba Y, Hiura K, Sugimoto M, Sato M, Kawahata H, Inaguma N, Kitamura Y And Nomura S. Role Of Osteopontin In Bone Remodeling Caused By Mechanical Stress. J Of Bone and Mineral Res. Nov 6, 1999.Vol 14.

73. Theill LE, Boyle WJ, Penninger JM. Rank-L and Rank: T Cells, Bone Loss, and Mammalian Evolution. Annu Rev Immunol 2002;20:795–823

74. Traianedes, K, Dallas, MR, Garrett, IR, Mundy and Bonewald LF. 5-Lipoxygenase Metabolites Inhibit Bone Formation In Vitro. Endocrinology. 1998;139:3178–3184

75. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science. 1996 Aug 2;273(5275):613-22

76. Wei S, Kitaura H, Zhou P, Ross Fp, Teitelbaum SL. Il-1 Mediates Tnf-Induced Osteoclastogenesis. J Clin Invest 2005. 115:282-290

77. Weitzmann NM, Pacifici R. Estrogen Deficiency and Bone Loss: An Inflammatory Tale. J Clin Invest. 2006;116(5):1186-1194

78. World Health Organization Study Group. Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis. Who Tech Rep Ser 1994;843:1-129.

79. Xin L, Zhang L, Wang N, Feng X, Liangjia B. Periodontal ligament remodeling and alveolar bone resorption during orthodontic tooth movement in rats with diabetes. Diabetes technology & therapeutics. 2010, 12(1): 65-73

80. Yamashita-Mikami E, Tanaka M, Sakurai N, Arai Y, Matsuo A, Ohshima H, Nomura S, Ejiri S. Correlations between Alveolar Bone Microstructure and Bone Turnover Markers in Pre- and Post-Menopausal Women. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Apr;115(4):E12-9.

81. Yamaguchi M. RANK/RANKL/OPG during orthodontic tooth movement. Orthodontics & Craniofacial Research, 2009. 12: 113–119.

82. Yavuzyilmaz E, Yamalik N, Bulut S, Ozen S, Ersoy F, Saatci U. The gingival crevicular fluid interleukin-1 beta and tumour necrosis factor-alpha levels in patients with rapidly progressive periodontitis. Aust Dent J 1995;40:46–9.

83. Gilbert S, Sunderland MA. Developmental Biology. 6th Edition. Sinauer Associates; 2000.

84. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast Differentiation Factor Is A Ligand For Osteoprotegerin/Osteoclastogenesis-Inhibitory Factor And Is Identical To Trance/Rankl. Proc Natl Acad Sci Usa 1998;95:3597–3602

85. Zallone A. Direct and Indirect Estrogen Actions on Osteoblasts and Osteoclasts. Ann N Y Acad Sci. 2006;1068:173-9

STATEMENT OF THESIS

The aim of this study is to determine the difference between pre and post-menopausal women receiving orthodontic treatment and determine if the changes after 24 hours of orthodontic activation between the groups are similar. It is hypothesized that there is no difference between pre and post-menopausal women receiving orthodontic treatment.

Differences in bone turnover markers in pre and post-menopausal women receiving orthodontic treatment

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Abstract

Objective: The aim of our study was to investigate the differences in bone turnover marker levels in pre and post-menopausal women receiving orthodontic treatment. **Methods:** Twenty-eight orthodontic patients were recruited from CWRU and faculty members' private practices. Subjects were screened and excluded according to the selection criteria. Sixteen control subjects (mean age 33.2 ± 8.4 years) and 12 post-menopausal (PM) subjects (mean age 63.4 ± 8.1 years) participated and their gingival crevicular fluid (GCF) was taken from 4 sites; 2 from the anterior and 2 posterior teeth. Samples were taken at pre orthodontic activation (T_0) and 24 hours after activation (T_1). GCF samples are stored in -80c and RANKL, osteopontin and ostonection level were investigated by ELISA. **Results:** All biomarker levels in Control and PM groups had similar increase of biomarker concentration after activation without statistical significant. At T_0, RANKL in PM was significantly lower than in control group (Control 6.74pg/ml± 5.45 vs PM 3.69 pg/ml± 3.52, p-value=0.035*), SPARC in PM was significantly higher than in control (Control 325.10pg/ml± 231.21 vs PM 872.8 pg/ml± 409.92, p-value= 0.026*) and OPN had no significant difference. **Conclusions:** Findings in our study suggest that although there is a difference in baseline bone turnover markers in PMW versus controls, there is no difference in the initial change of these biomarkers in PMW vs. controls. This suggests that although underlying bone differences between these groups may exist in periodontally healthy subjects, orthodontic activation does not trigger an exaggerated response in PMW.

Introduction

According to the American Association of Orthodontics (AAO), number of adult patients increased 14 percent from 2010 to 2012, a record high of 1.2 million patients age 18 and older. According to the AAO's survey, 56 percent of the adult patients were female. Practices across the nation are experiencing higher number of female adult patients. Orthodontic tooth movement is based on bone remodeling. As post-menopausal women seek orthodontic treatment, their treatment time, outcome, oral health and sub sequential bone loss becomes a concern. Adult women face a major risk in bone loss and subsequent fracture as they enter menopause[1, 2]. At post menopause, bone remodeling rates double and triple 13 years later. They also remain elevated in osteoporosis. This change contributes to increases in age-related skeletal fragility in women[3]. As estrogen level becomes deficient, micro-architectural deterioration of bone tissue and low bone mass can occur. This is characteristics of osteoporosis[7]. Dual energy X-ray absorptiometry (DXA) is the most reliable way to determine bone mineral density (BMD), which is one of the major risk factors for fractures from osteoporosis[4]. Currently, only a few BMD measurements are taken among patients in high-risk groups because of no symptoms appearing prior to fractures[5]. A large proportion of postmenopausal women with a high risk of fractures from osteoporosis still remain undiagnosed[6]. About 54 million Americans have osteoporosis and low bone mass, placing them at increased risk for osteoporosis. Several researches also linked osteoporosis to be a risk factor for tooth loss and reduced mandibular bone mass and density[8, 9.] The World Health Organization

(WHO) has established criteria for making the diagnosis of osteoporosis, as well as determining levels that predict higher chances of fractures. These criteria are based on comparing the bone mass density (BMD) of the patient with that of a typical healthy, young female's. BMD values that fall well below the average for the healthy, young females (stated statistically as 2.5 standard deviations below the average) are diagnosed as osteoporotic. Many women over 50 years of age still have not had adequate screening and testing for osteoporosis. Moreover, knowledge of changes in healthy, periodontitis free post-menopausal females are not fully understood when orthodontic forces are applied.

Bone turnover is triggered by local and systemic factors. In orthodontic treatment, pressure and tension force can stimulate local factors to trigger the bone remodeling cascade. A receptor activator of NFkB (RANK) is a type I protein present on the cell surface of osteoclasts precursor cells. When stimulated, osteoblasts up-regulate expression of RANK Ligand (RANKL), which binds to RANK, activating signaling pathways that promote osteoclast differentiation and survival[10, 11]. RANKL is produced by osteoblasts and is one of many signaling molecules that facilitate cross-talk between the osteoblasts and osteoclasts and help coordinate bone remodeling[12]. Osteoprotegerin (OPG), also released by osteoblasts, can also bind to RANKL, acting as a competitive substrate to prevent RANK and RANKL from coming in contact. Inhibitors of RANKL have also shown as potential treatment for osteoporosis in humans.

Osteopontin (OPN) is a major non-collagenous bone matrix protein; it contains a motif of glycine-arginine-glycine-aspartic acid-serine (GRGDS). This 314 amino acid sequence promotes call attachment via cellular $\alpha_v\beta_3$ integrin. OPN have been suggested to enhance or regulate the adhesion, attachment, and spreading of osteoclasts to the bone surface during resorption. OPN is produced by osteoblasts and osteoclasts and plays a vital role in bone remodeling[13]. OPN expressed in osteocytes and osteoblastic cells due to mechanical stress might induce migration of osteoclastic precursor cells to the bone surface and promote bone resorption. These results indicate that these cells responded to mechanical stress loaded on the bone with expression of the osteopontin gene. Several studies showed OPN increasing after 12 hours of applying mechanical force[14]. OPN is possibly a multifunctional protein that acts as a coupling factor for bone resorption and formation in the process of bone remodeling.

Osteonectin or secreted protein acidic and rich in cysteine (SPARC) is the most abundant non-collagenous extracellular matrix protein in bone[15]. Analysis of the skeletal phenotype of SPARC-null mice has provided excellent information on the function of this protein in bone. SPARC-null mice develop profound low-turnover that becomes progressively worse with age[16]; they also found decreased numbers of osteoblasts and osteoclasts, and a markedly decreased bone-formation rate. In the skeleton, osteonectin promotes osteoblast commitment, suppresses adipogenesis, and regulates the balance between bone formation and resorption in response to PTH treatment. It is highly expressed early in osteoblastic differentiation, but its

expression decreases as the cells acquire characteristics of mature osteoblasts. SPARC-null mice have decreased trabecular bone volume due to decreased trabecular number. Boskey et al concluded in 2003 that bone resorption rate becomes higher than bone forming and result in reduced bone mass due to lack of osteonectin[16].

The aim of this study is to investigate the difference in bone turnover markers in gingival crevicular fluid between pre and post–menopausal women receiving orthodontic treatment.

Materials and Method

Subjects

Our study recruited adult females (21 and over) subjects having plaque score of 80% clean or better, with no current periodontal disease and having more than 4 incisors and 4 premolars present from patients undergoing orthodontic treatment at Orthodontic Clinic, Case Western Reserve University (CWRU) and from private practice of faculty members, Cleveland, Ohio. This prospective cohort single blinded study has been approved by CWRU institutional review board (IRB# 2012196). Subjects were excluded based on the criteria shown in Table1.

Patients are then asked to answer questions regarding their history of menstrual cycle, medication, general health history and current oral health history.

Of the 110 patients that were screened, 28 fit the criteria and signed informed consent (appendix a) to participate. Those having not had a period in 12 months or

more were designated postmenopausal group the rest were considered pre-menopausal and designated control group.

Once consent form is signed, patients are subject to collect gingival crevicular fluid (GCF) by one of two examiners (S.S. and C.J.).

Total of 16 subjects were included in the pre-menopausal group (control) and 12 in the post-menopausal group. Four samples are collected and pooled from each subject to represent the oral condition rather than to be site specific

GCF collection protocol: Molars and canines are not included as they have higher variability in inflammation and are subject to larger force in orthodontic in cases that have rubber band wear or retraction/protraction. Four sites are randomly selected by using the distolabial suface of 2 incisors and distobuccal surface of 2 premolars from the same arch. Our study used method of collecting GCF according to Lamster et al in 1987[39]. Method in collecting GCF is as followed: The sites are isolated with cotton rolls and are slightly air dry or wiped with gauze. Periopaper™ is placed in the periodontal pocket and inserted to the attached tissue with the aid of periodontal probe. Periopaper™ is left to accumulate GCF for at least 40 seconds (Figure 1). Each strip is then transferred to an Eppendorf safe-lock tube and store in thermos containing dry ice, which was tested to hold the temperature under -50c for longer than 6 hours (Smuthkochorn-Jones Thermos, Figure 2). Once all four strips are collected and labeled, the samples are transported in the Smuthkochorn-Jones thermos to a -80c refrigerator within 4 hours. All samples are then transferred to the CWRU lab and then diluted and run to find the concentration of the following

proteins: RANKL, osteopontin (OPN) and osteonectin (SPARC). RANKL is assessed using Multiplex enzyme-linked immunosorbent assay (ELISA), osteopontin is measured by ELISA test and osteonectin uses SPARC analysis.

Statistical analysis

Sample size calculation[17]:

If MU 1 (control) = 140 pg inflam/µl overall volume

if MU 2 (experimental) = 160 pg inflam marker/µl overall volume

if sigma value (std. dev.) = 27 pg inflam marker/ µl overall volume

then ideal sample size = 15 subjects, power level 0.80 and $p < .05$

Thus, for $\alpha<.05$ and a desired power of 0.80, the ideal sample size for each sample separately is 15 subjects per group. To increase the strength of the study, we will attempt to garner as many subjects as possible per group.t-test will be used to compare means of each subject's data by comparing pre and post activation to validate the activity of bone turn over markers. Another t-test to investigate the differences between the experimental and control group will then be carried out. All statistical calculations will be performed with SPSS (version 22). Significance to be accepted for analysis will be $p< .05$.

RESULT

Sixteen subjects were recruited into our control group and 12 in post-Menopausal group. The demographic data of the subjects are described in table 2, it consists of 10 Caucasians, 2 African-American, 4 Asian (3 Indians and 1 Chinese) in

the control group and 10 Caucasians and 2 African-American in the post-Menopausal group.

In the control group, 2 subjects did not return to have the GCF collected after 24 hour and was not used in finding the changes after orthodontic activation and post-menopausal measurements.

All the pre and post-menopausal measurement for OPN, RANKL and SPARC are not normally distributed. The changes in the pre vs. post activation for both groups are normally distributed. Using the Mann Whitney U test to compare the change between pre and post activation in pre and post-menopausal group, we found that all three proteins had no significant difference in the change before and 24 hour after activation.

RANKL (Figure 3)

When comparing the baseline (T_0) between pre and post-menopausal subjects, Wilcoxon t-test showed that there is significant difference between the pre and post-menopausal groups (P value=0.035*). The control group had a mean of 6.74 pg/µl $\pm$ 5.45 and post-menopausal group had 3.69 pg/µl. $\pm$SD 3.52 ($p<0.05$). RANKL level in post-menopausal women is lower than in pre-menopausal women.

In control group, the changes in T_0 versus T_1 showed significant increase with T_0 6.74 pg/ml $\pm$ 5.45 and T_1 11.94 pg/ml $\pm$ 7.66 and the average change of 6.58 pg/ml $\pm$ 5.11 (P value=0.041*).

In post-menopausal group, the changes in pre and post activation of orthodontic adjustment showed significantly increased change (P value=0.000*) in all

the subjects, T_0 3.69 pg/ml ± 3.52 and T_1 10.05 pg/ml ± 5.04 and with the average change of 6.36 pg/ml ±4.24.

When comparing the changed in pre and post-menopausal group, our study showed that there is no statistical difference (P value=0.777) in the change between the control (T_1-T_0 6.58 pg/ml ±5.11) and post-menopausal groups (T_1-T_0. 6.36 pg/ml±4.24).

Osteopontin (OPN) (Figure 3)

Osteopontin level in pre and post-menopausal groups prior to activation did not show statistically significant difference (p value=0.419) with means of 282.36 pg/ml ±149.8 and 342.26 pg/ml ± 149.8 respectively

Changes in osteopontin in pre and post activation of orthodontic adjustment had statistically significant difference (p value=) In control group, the changes in T_0 versus T_1 showed significant increase with T_0 278.62 pg/ml ± 138.9 and T_1 531.72 pg/ml ± 465.98 (P value=0.031*)

In post-menopausal group, the changes in pre and post activation of orthodontic adjustment showed significantly increased change at T_0 323.26 pg/ml ± 157.27 and T_1 489.61 pg/ml ± 268.65 (P value=0.006*)

When comparing the changed in pre and post-menopausal group, our study showed that there is no statistical difference (p value=0.269) in the change between the pre (T_1-T_0 292.81 pg/ml± 454.42) and post-menopausal groups (T_1-T_0. 166.35 pg/ml± 170.44)

Osteonectin (Figure4)

When comparing the baseline (pre activation) between control and post-menopausal subjects, Mann-Whitney U test showed that there is significant difference between the pre and post-menopausal groups (p value=0.026*). The pre- menopausal group had a mean of osteonectin level of 325.10 pg/ml ±231.21 and post-menopausal group had 872.8 pg/ml ±409.92.

In control group, SPARC concentration in pre and post activation of orthodontic adjustment had statistically significant difference (p value=). In control group, the changes in T_0 versus T_1 showed significant increase with T_0 325.1 pg/ml ± 231.21 and T_1 872.8 pg/ml ± 409.92 (P value=0.006*)

In post-menopausal group, the changes in pre and post activation of orthodontic adjustment showed significantly increased change at T_0 558.83 pg/ml ± 362.80 and T_1 1061.77 pg/ml ± 822.85 (P value=0.006*)

When comparing the change in pre and post-menopausal group, our study showed that there is no statistical difference (p value=0.446) in the change between the pre (T_1-T_0 503.26 pg/ml± 391.85) and post-menopausal groups (T_1-T_0. 547.05 pg/ml± 569.96)

Discussion

This study was designed to evaluate the gingival crevicular fluid concentration of RANKL, Osteopontin and Osteonectin before orthodontic activation and 24 hours after activation. Tension and pressure sides were randomly selected as this is a pilot study to investigate initial activities of bone turnover markers in post-menopausal

women. From previous studies, both pressure and tension sides have increased of biomarkers activities in the first 24 hours[18]. Only buccal and labial surfaces were selected due to its convenience.

RANKL

When periodontium is healthy, only a negligible amount of GCF is produced. On the other hand, an increased presence of inflammation can significantly increase GCF production and secretion. In orthodontic tooth movement, an increased in GCF is caused by increased plaque retention[19] and acute generalized inflammation reaction in the periodontal tissue. Production of GCF will have increased bone turnover markers level that is not produced by bacterial invasion. Good oral hygiene is encouraged to reduce false positive of bone resorption markers. Many reported conflicting findings in RANKL and OPG, as they are usually inversed. Reports of not able to detect any OPG and a very low level of RANKL and the other way around is not uncommon. In this study, RANKL is the main focus. RANKL in pre-menopausal group is higher than in post-menopausal group before the activation of orthodontic movement. Results from several researches suggest that serum RANKL concentrations were lower than in pre-menopausal women and unrelated with bone mineral density, the age- and menopause- dependent changes of serum OPG and RANKL might be a protective mechanism against the accelerated bone loss in postmenopausal women[20,21]. As there is no report of average concentration of RANKL in human GCF and this level changes constantly over time, changes in the level of each individual is the focus of our study. Post and pre-menopausal groups

had similar change 24 hours after activation of orthodontic force is applied. We can assume that both groups have similar initial response from orthodontic force. With the subject under actual treatment, forces are randomly selected and controlled force was not used. This can be positive and negative. As we treat patients, we do not always have full control on the exact amount of force applied to each tooth, let alone the action-reaction of continuous arch wire mechanic. From randomly selecting sites to measure RANKL, we would most likely find actual response from actual treatment being treated. Negative side is the loss of control in variable and loss of ability to predict response to force, if there is one. Increased RANKL level can demonstrate bone remodeling. Unbalanced level of RANKL can illustrate over activation of bone resorption. Our result did not show any highly increased level that differed from the pre-menopausal group and it may reduce limitation to treating post-menopausal women. With limited number of subjects, it is difficult to assume if this result can be a true representation of the population. However, the trend that is unanimous in the increase of RANKL level after activation can be inferred that post-menopausal women have similar respond to pre-menopausal women 24 hour after orthodontic force is applied. As RANKL is highly involved in bone remodeling further study can confirm that bone loss in post-menopausal women from orthodontic treatment has no significant differences than in pre-menopausal women.

Osteopontin and osteonectin

Osteopontin and osteonectin are non-collagenous protein expressed mainly in the PL fibers. As orthodontic force is applied, the fibers are immediately stretched.

Cementoblast and PL fibers expressed these proteins as they are remodeling. Although osteopontin and osteonectin concentrations peak at day 7 and 14, respectively, our study showed that an increased can be detected as early as 24 hours after activation. Osteonectin showed significantly higher baseline value in pre-menopausal than in post-menopausal. However, the changes in both osteopontin and osteonectin are similar in pre-and post-menopausal groups. There were no significant difference in baseline value of pre and post-menopausal women in osteopontin level. In congruent to other researches, osteonectin had higher expression after 24 hours of activation. Osteopontin had lower expression and does not have significant differences between the two groups. Bone loss can be initiated by attachment loss, PL fiber also play an important role in this balance. As osteonectin and osteopontin are both also expressed in the bone and PL fibers, similar trends of the levels after orthodontic activation ensures treatment provider of similar side effects.

Limitations and future study

Our study did not have the sufficient amount of samples due to limitation of time, funding and highly selective exclusion criteria to reduce errors. Future research can eliminate these problems by increasing research time to recruit more subjects. Eliminating bias from oral hygiene (reduce plaque accumulation) and consistency in sample collection is key to getting a true representation of biomarker levels. Our study found an error in communicating with the laboratory that ran our samples. Labeling became an issue and data had to be matched by hand to match with the original assignments. Following the subjects for more than 24 hours would have

given a more concrete conclusion of the response in bone turnover markers as it may peaked later than the control group and/or more or less.

Conclusion

This initial study to compare pre and post-menopausal women under orthodontic treatment has shed some light on the similarity of the response found after force is applied. Speculation from reduced estrogen and axial bone has made treating post-menopausal women questionable in terms of increase side effects such as bone loss, attachment loss and time management. Our study have shown that, in the absent of bacterial assault, despite some differences in the baseline levels, post-menopausal women react to orthodontic force similar to pre-menopausal women.

Figures



Figure 1. GCF collection: Teeth are wiped with gauze prior to any cleaning from the patient and moisture is controlled with cotton rolls. Periopaper™ is gently inserted into the sulcus to absorb the GCF.



Figure 2. Smuthkochorn-Jones Thermos

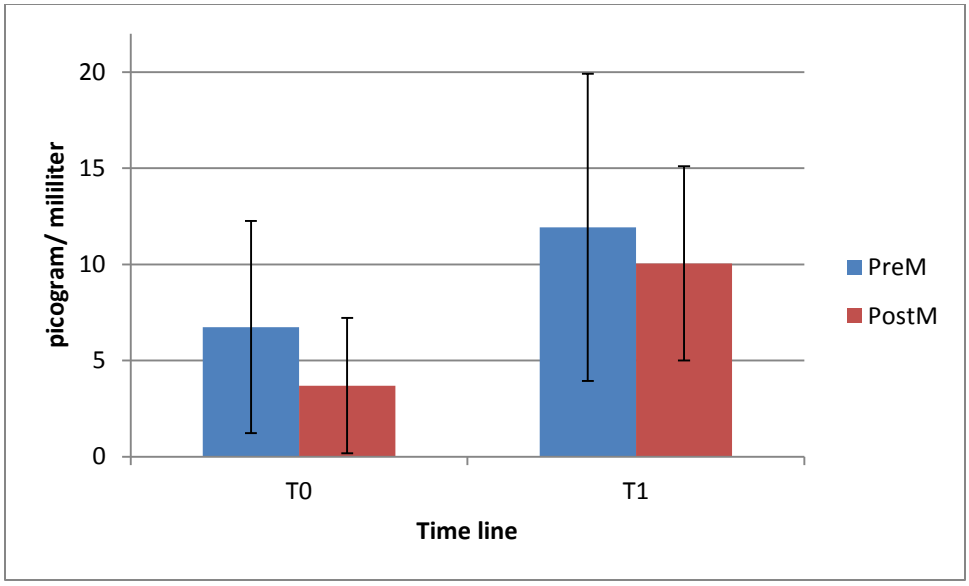


Figure 3. RANKL concentration in pre and post-menopausal group comparing pre and 24 hour after activation.

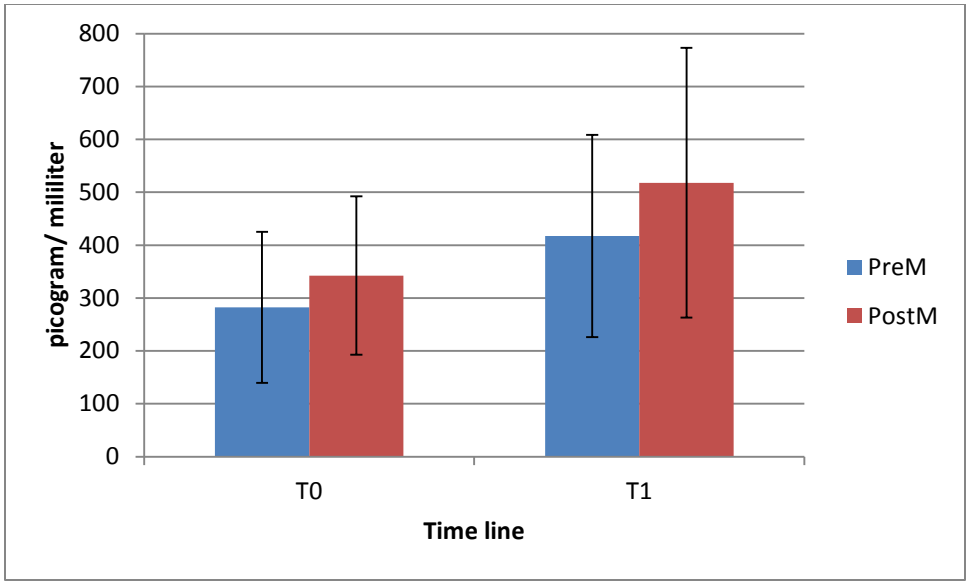


Figure 4. Osteopontin concentration in pre and post-menopausal group comparing pre and 24 hour after activation.

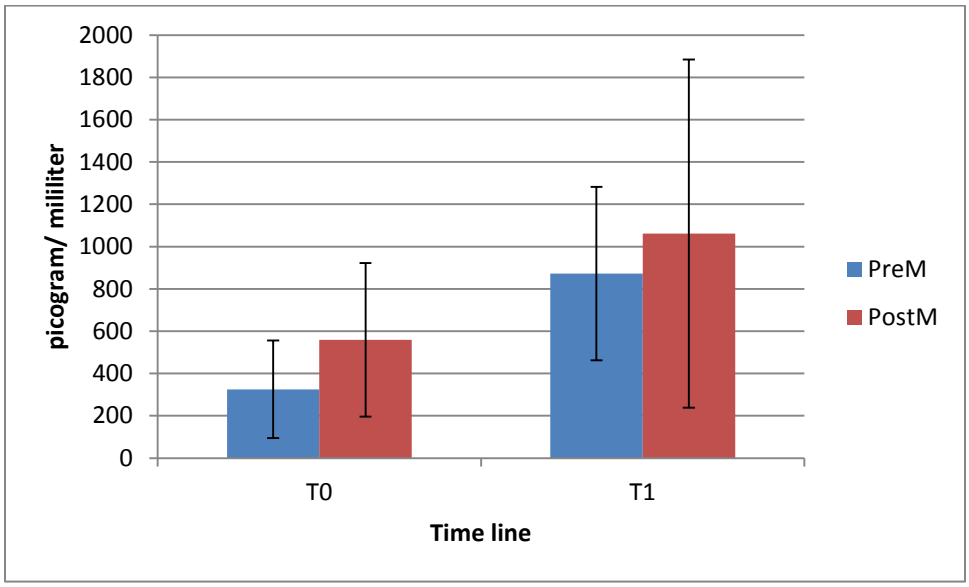


Figure 5. Osteonectin concentration in pre and post-menopausal group comparing pre and 24 hour after activation.

Tables

Exclusion criteria	Exclude
Medication (known to modulate bone turnover)	-under anti-inflammatory medication: NSAIDs, Corticosteroids -Bisphosphonate -Hormone therapy -Prolong use of Calcium and vitamin D (longer than 1 year)
Medical history (based on patient report	Has following condition or disease: rheumatoid, diabetics, Crohn's disease and other inflammatory disease
Dental history (based on patient report)	-had orthognathic surgery or corticotomy within 6 months -is using any accelerated device for treatment time reduction
Menstrual cycle	Intermittent menstruation or ceased of menstruation for less than 1 year

Table 1. Exclusion criteria

	Pre-menopausal n = 16	Post-menopausal n=12
Ethnicity	White = 10 African-American= 2 Asian = 1 Indian= 3	White = 10 African-American = 2
Age (average, range)	32, (23-47)	63, (56-80)

Table 2. Demographic data and age of subjects

References

1. Cheung, Chaudhry R, Kapral M, Jackevicius C, Robinson G. BMC Women's Health. 2004; 4(Suppl 1): S23
2. Kai Luo, Souzhi Ma, Jianbin Guo, Yongling Huang, Fuhua Yan, Yin Xia. Association between Postmenopausal Osteoporosis and Experimental Periodontitis. BioMed Research International. Volume 2014, Article ID 316134, 2014.
3. French DL, J. M. Muir, and C. E. Webber, The ovariectomized, mature rat model of postmenopausal osteoporosis: an assessment of the bone sparing effects of curcumin, Phytomedicine, vol. 15, no. 12, pp. 1069–1078, 2008.
4. Lerner UH. Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. J Dent Res. 2006 Jul;85(7):596-607.
5. Miller SC, B. M. Bowman, and W. S. S. Jee, "Available animal models of osteopenia—small and large," Bone, vol. 17, no. 4, pp. 117–123, 1995.
6. Payne JB, Reinhardt RA, Nummikoski PV, Patil KD. Longitudinal alveolar bone loss in postmenopausal osteoporotic/osteopenic women. Osteoporos Int. 1999;10(1):34-40.
7. Yamashita-Mikami E, Tanaka M, Sakurai N, Arai Y, Matsuo A, Ohshima H, Nomura S, Ejiri S. Correlations between alveolar bone microstructure and bone turnover markers in pre- and post-menopausal women. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Apr;115(4):e12-9.

8. World Health Organization Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. WHO Tech Rep Ser 1994;843:1-129.
9. Jin N, Lin S, Zhang Y, Chen F. Assess the discrimination of Achilles Insight calcaneus quantitative ultrasound device for osteoporosis in Chinese women: compared with dual energy X-ray absorptiometry measurements. Eur J Radiol 2010;76: 265-8
10. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast Differentiation Factor Is A Ligand For Osteoprotegerin/Osteoclastogenesis-Inhibitory Factor And Is Identical To Trance/Rankl. Proc Natl Acad Sci Usa 1998;95:3597–3602
11. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93:165–176
12. Theill LE, Boyle WJ, Penninger JM. RANK-L and RANK: T cells, bone loss, and mammalian evolution. Annu Rev Immunol 2002;20:795–823
13. Takano-Yamamoto T, Takemura T, Kitamura Y And Nomura S. Site-Specific Expression Of Mrnas For Osteonectin And Osteopontin Revealed By In Situ

Hybridization In Rat Periodontal Ligament During Physiological Tooth Movement. J Histochem Cytochem. 1994, 42:885.

14. Terai K, Takeno-Yamamoto T, Ohba Y, Hiura K, Sugimoto M, Sato M, Kawahata H, Inaguma N, Kitamura Y and Nomura S. Role of Osteopontin in Bone Remodeling Caused by Mechanical Stress. J of Bone and Mineral Res. Vol 14. Nov 6, 1999.
15. Delany AM, Hankenson KD. Thrombospondin-2 and SPARC/osteonectin are critical regulators of bone remodeling. J Cell Commun Signal. 2009 Dec;3(3-4):227-38.
16. Boskey AL, Moore D, Amiling M, Canalis E, Delany AM. Infrared Analysis of the Mineral and Matrix in Bones of Osteonectin-Null Mice and Their Wildtype Controls. J of Bone and Min Res. 2003. Vol 18, No. 6.
17. Faulkner MG. Gingival Crevicular Fluid (GCF) levels of Interleukin-6 (IL-6), SGP 130 and sIL-6R during orthodontic tooth movement. Master's thesis. UNLV 2011.
18. Hart KA. RANKL and OPG levels in Response to Orthodontic Forces. Master's thesis. Uni of Tenn. 2012.
19. Sakellari D, Menti S. and Konstantinidis A. Free soluble receptor activator of nuclear factor-κb ligand in gingival crevicular fluid correlates with distinct pathogens in periodontitis patients. Journal of Clinical Periodontology, 2008. 35: 938–943.

20. LaCroix AZ, Jackson RD, Aragaki A, Kooperberg C, Cauley JA, Chen Z, Leboff MS, Duggan D, Wactawski-Wende J OPG and sRANKL serum levels and incident hip fracture in postmenopausal Caucasian women in the Women's Health Initiative Observational Study. Bone. 2013 Oct;56(2):474-81.
21. Liu JM, Zhao HY, Ning G, Zhao YJ, Chen Y, Zhang Zh, Sun LH, Xu MY, Chen JL. Relationships between the changes of serum levels of OPG and RANKL with age, menopause, bone biochemical markers and bone mineral density in Chinese women aged 20-75. Calcif Tissue Int. 2005 Jan;76(1):1-6.
22. Eastell R, Blumsohn A. The value of biochemical markers of bone turnover in osteoporosis. J Rheumatol 1997;24:1215-7.
23. Singer FR, Eyre DR. Using biochemical markers of bone turnover in clinical practice. Cleve Clin J Med 2008;75:739-50
24. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res 2000;15:1526-36
25. Sidiropoulou-Chatzigiannis S1, Kourtidou M, Tsalikis L. The effect of osteoporosis on periodontal status, alveolar bone and orthodontic tooth movement. A literature review. J Int Acad Periodontol. 2007 Jul;9(3):77-84.
26. Gilbert SF, Sunderland MA. Developmental Biology. 6th edition.: Sinauer Associates; 2000

27. Carranza FA, Newman MG, Takei HH, Klolevld PR. Carranza's Clinical Periodontology , 12th edition published by Elsevier
28. Raisz LG. Physiology and Pathophysiology of Bone Remodeling. Clinical Chemistry August 1999 vol. 45 no. 8 1353-1358
29. Xin Li, M,Lin Zhang, Na Wang, Xiaohong F, and Liangjia B. Periodontal Ligament Remodeling and Alveolar Bone Resorption During Orthodontic Tooth Movement in Rats with Diabetes. DIABETES TECHNOLOGY & THERAPEUTICS. Volume 12, Number 1, 2010.
30. Mabuchi R, Matsuzaka K, Shimono M: Cell proliferation and cell death in periodontal ligaments during orthodontic tooth movement. J Periodontal Res 2002;37:118–124
31. Dempster DW, Cosman F, Parisien M, Shen V. Anabolic actions of parathyroid hormone on bone. Endocr Rev 1993;14:690-709
32. Rosen CJ, Donahue LR. Insulin-like growth factors and bone—the osteoporosis connection revisited. Proc Soc Exp Biol Med 1998;219:1-7.
33. Advani S, LaFrancis D, Bogdanovic E, Taxel P, Raisz LG, Kream BE. Dexamethasone suppresses in vivo levels of bone collagen synthesis in neonatal mice. Bone 1997;20:41-46.
34. Pacifici R. Cytokines, estrogen and postmenopausal osteoporosis—the second decade. Endocrinology 1998;139:2659-2661
35. Raisz LG. The osteoporosis revolution. Ann Intern Med 1997;126:458-462.

36. Riggs BL, Khosla S, Melton LJ. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 1998;13:763-773.
37. Prestwood KM, Pilbeam CC, Raisz LG. Treatment of osteoporosis. Annu Rev Med 1995;46:249-256.
38. Eastell R. Treatment of postmenopausal osteoporosis. N Engl J Med 1998;338:736-746.
39. Lamster IB, Harper DS, Fiorello LA, Oshrain R, Celenti RS and Gordon JM. Lysosomal and cytoplasmatic enzyme activity, crevicular fluid volume, and clinical parameters characterizing gingival sites with shallow to intermediate probing depths. *J Periodontol.* 1985;58:614–621.
40. Riggs BL, Khosla S, Melton LJ 3rd. Endocr Rev. Sex steroids and the construction and conservation of the adult skeleton. 2002 Jun; 23(3):279-302.
41. Crockett JC, Rogers MJ, Coxon FP, Hocking LJ, Helfrich MH. Bone remodeling at a glance. April 1, 2011 J Cell Sci 124, 991-998.
42. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest. 2006;116(5):1186-1194
43. Lindsay R. Prevention and treatment of osteoporosis. Lancet. 1993;341:801-805.
44. Lips P. Vitamin D physiology. Prog Biophys Mol Biol. 2006;92(1):4-89

45. Zallone A. Direct and indirect estrogen actions on osteoblasts and osteoclasts. Ann N Y Acad Sci. 2006;1068:173-9

46. Bonnelye, E, and Aubin, JE. Estrogen receptor-related receptor alpha: a mediator of estrogen response in bone. J. Clin. Endocrinol. Metab. 2005. 90:3115–3121

47. Takayanagi H. Mechanistic insight into osteoclast differentiation in osteoimmunology. J Mol Med. 2005;83(3):170-9

48. Pilbeam, CC, Harrison, JR, Raisz, L.G. Prostaglandins and bone metabolism. In Principles of bone biology. Academic Press. San Diego, California, USA. 2002. 979–994

49. Traianedes K., Dallas MR., Garrett IR., Mundy GR, and Bonewald LF. 5-Lipoxygenase metabolites inhibit bone formation in vitro. Endocrinology. 1998;139:3178–3184

50. Dewhirst FE, Stashenko PP, Mole JE, Tsurumachi T. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1 beta. J Immunol 1985.135:2562-2568.

51. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature. 1986 319:516-518

52. Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 1990. 145:3297-3303

53. Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, Kojima K. Interleukin 1 beta, interleukin 6, beta 2-microglobulin, and transforming growth factor-alpha in gingival crevicular fluid from human periodontal disease. Arch Oral Biol 1999. 44:535-539

54. Graves DT and Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol 2003.74:391-401

55. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest 2000. 106:1481-1488

56. Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced osteoclastogenesis. J Clin Invest 2005. 115:282-290

57. Jacobs C R, Temiyasathit S. and Castillo AB. Osteocyte mechanobiology and pericellular mechanics. Annu. Rev. Biomed. Eng. 2010. 12, 369-400.

58. Newald B and Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone. 2008. 42, 606-615.

59. Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K. Levels of RANKL and OPG in Gingival Crevicular Fluid During Orthodontic Tooth Movement and Effect of Compression Force on Release from Periodontal Ligament Cells in vitro. Orthod Craniofac Res. 2006. May; 9(2):63-70

60. Cardoso L, Herman BC, Verborgt O, Laudier D, Majeska RJ, Schaffler MB. Osteocyte apoptosis controls activation of intracortical resorption in response to bone fatigue. J. Bone Miner. Res. 2009. 24, 597-605.
61. Dimitrios j. Hadjidakis and ioannis i. Androulakis. Bone Remodeling. Ann. N.Y. Acad. Sci. 2006. 1092: 385–396.
62. Graber TM, Vanarsdall RL, Vih KWL. Orthodontics, Current principles and Techniques, 4th Edition. St. Louis. Mosby. 2005
63. Nayak BN, Galil KA, Wiltshire W and Lekic PC. Molecular Biology of Orthodontic Tooth Movement. J Dent Oral Health 2013 Vol 1: 101
64. Masella RS, Meister M. Current concepts in the biology of orthodontic tooth movement. Am J of Orthod and Dentofacial Orthop. Volume 129, Number 4. 2005
65. Storey E. The nature of tooth movement. Am J Orthod 1973;63:292–314
66. Davidovitch Z, Nicolay OF, Ngan PW, Shanfeld JL. Neurotrans-mitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dent Clin North Am 1988;32:411–35
67. Shiotani A, Shibasaki Y, Sasaki T. Localization of receptor acti-vator of NF-kappaB ligand, RANKL, in periodontal tissues during experimental movement of rat molars.J Electron Microsc 2001;50:365–9
68. Aihara N, Otsuka A, Yamaguchi M, Okada H, Utunomiya T, Yamamoto H et al. Localization of RANKL and cathepsin K, B, and L in rat periodontal tissues during experimental tooth movement. Orthod Waves 2005;64:107

69. Kim T, Handa A, Iida J, Yoshida S. RANKL expression in rat periodontal ligament subjected to a continuous orthodontic force. Arch Oral Biol 2007;52:244–50

70. Katagiri T, Takshashi N. Regulatory mechanisms of osteoblast and osteoclast differentiation. Oral Dis. 2002 May;8(3):147-59.