An in Vitro Evaluation of the Expression of IL-1β and TNF-α in Human Periodontal Ligament Fibroblasts after Exposure to Mechanical Vibration Combined with Compressive Stress

Buntarika Unat

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Oral Health Sciences
Prince of Songkla University
2017

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Major Program	Oral Health Sciences

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

....Signature

(Miss. Buntarika Unat)

Candidate

ชื่อวิทยานิพนธ์ การประเมินผลการแสดงออกของอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟาในเซลล์เนื้อเยื่อปริทันต์ของมนุษย์ ภายหลังจากได้รับแรงสั่นสะเทือนทางกลร่วมกับแรงกด
ผู้เขียน นางสาวบุณฑริกา อู่นาท
สาขาวิชา วิทยาศาสตร์สุขภาพช่องปาก
ปีการศึกษา 2559

บทคัดย่อ

การใช้แรงสั่นสะเทือนเพื่อนำมาเร่งการเคลื่อนที่ของฟัน เป็นวิธีการหนึ่งที่ได้รับความสนใจเป็นอย่างมาก แต่อย่างไรก็ตามการศึกษาเกี่ยวกับผลของแรงสั่นสะเทือนต่อการเร่งการเคลื่อนที่ของฟันยังคงเป็นที่ขัดแย้ง นอกจากนี้กลไกของแรงสั่นสะเทือนในการกระตุ้นการเคลื่อนที่ของฟันยังไม่เป็นที่ทราบแน่ชัด **วัตถุประสงค์** เพื่อวัดผลของแรงสั่นสะเทือนร่วมกับแรงกดในเซลล์เนื้อเยื่อปริทันต์ของมนุษย์ ผ่านทางการแสดงออกของยีนและโปรตีนอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟา **วิธีการวิจัย** นำเซลล์เนื้อเยื่อปริทันต์ของมนุษย์จากฟันกรามน้อยซี่ที่ 1 จากนั้นแบ่งกลุ่มโดยการสุ่มทั้งหมด 6 กลุ่มดังนี้ กลุ่มควบคุม (CT; กลุ่มที่ไม่ได้รับแรงใดๆ) กลุ่มที่ได้รับแรงกด (COM; 2 g/cm^2) กลุ่มที่ได้รับแรงสั่นสะเทือน (Vi; ความถี่ 30 Hz & 60 Hz) และกลุ่มที่ได้รับแรงสั่นสะเทือนร่วมกับแรงกด (CV) เทคนิคการเพิ่มขยายกรดนิวคลีอิคถูกนำมาใช้ในการวัดการแสดงออกของยีนอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟา และปฏิกิริยาที่เฉพาะเจาะจงของแอนติบอดี้และแอนติเจนโดยใช้เอนไซม์ถูกนำมาวิเคราะห์ระดับโปรตีนของอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟา **ผลการศึกษา** การแสดงออกของยีนอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ชนิดอัลฟาเพิ่มขึ้นอย่างมีนัยสำคัญในกลุ่มที่ได้รับแรงกด และกลุ่มที่ได้รับแรงสั่นสะเทือนร่วมกับแรงกดเมื่อเทียบกับกลุ่มที่ไม่ได้รับแรงใดๆ และสูงสุดในกลุ่มที่ได้รับแรงสั่นสะเทือนร่วมกับแรงกดที่ความถี่ 30 Hz ในขณะที่การแสดงออกของยีนอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟาลดลงอย่างมีนัยสำคัญในทุกกลุ่มของกลุ่มที่ได้รับแรงสั่น และมีค่าน้อยกว่ากลุ่มที่ไม่ได้รับแรงใดๆ นอกจากนี้ยังพบว่าระดับของโปรตีนอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์

เนโครซิส แฟกเตอร์ ชนิดอัลฟาในกลุ่มที่ได้รับแรงกด และกลุ่มที่ได้รับแรงสั่นสะเทือนร่วมกับแรงกดมีค่าสูงกว่ากลุ่มที่ไม่ได้รับแรงใดๆ แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญ **สรุป** แรงสั่นสะเทือนร่วมกับแรงกดสามารถเปลี่ยนแปลงการแสดงออกของยีนอินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟา แต่ไม่เปลี่ยนแปลงการแสดงออกของโปรตีน อินเตอร์ลูคิน 1 ชนิดเบต้าและทูเมอร์ เนโครซิส แฟกเตอร์ ชนิดอัลฟา ในเซลล์เนื้อเยื่อปริทันต์ของมนุษย์ได้

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ABSTRACT

The use of vibration to accelerate the tooth movement is very interesting. However, the studies on the effect of vibration on accelerated tooth movement still remain controversial. Moreover, the mechanisms of mechanical vibration to stimulate the tooth movement are not known. **Objectives:** To investigate the effects of mechanical vibration combined with the compressive force in human PDL cells via the gene and protein expression of IL-1β and TNF-α. **Materials and Methods:** Human PDL cells derived from first premolars were randomized into six groups: CT groups (unloaded), COM groups (compressive stress 2 g/cm^2), Vi groups (vibration: 30 Hz and 60 Hz), and CV groups (compression & vibration). Real-time PCR was used to investigate the gene expression of IL-1β and TNF-α and ELISA was used to quantitate the protein levels of IL-1β and TNF-α. **Results:** The expression of IL-1β and TNF-α in COM and CV groups (30 & 60 Hz) increased significantly when compared with CT groups and highest expression in CV group of 30 Hz. While the expression of IL-1β and TNF-α decreased in all Vi groups and was less than the control group. Furthermore, the protein levels of IL-1β and TNF-α in COM groups and all CV groups were higher than CT group but not significant. **Conclusions:** The mechanical vibration combined with compressive stress can alter only the gene expression of IL-1β and TNF-α but does not change the protein expression of IL-1β and TNF-α in human PDL cells.

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Buntarika Unat

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LIST OF ABBREVIATION AND SYMBOLS

PDL = Periodontal ligament

RANK = Receptor activator of nuclear factor kappa B

RANKL = Receptor activator of nuclear factor kappa B ligand

OPG = Osteoprotegerin

M-CSF = Macrophage colony-stimulating factor

IL-1β = Interleukin 1-beta

TNF-α = Tumor necrosis factor – alpha

PGE = Prostaglandin E

GCF = Gingival crevicular fluid

LMHF = Low magnitude high frequency

ECM = Extracellular matrix

DMEM = Dulbecco’s modified essential medium

FBS = Fetal bovine serum

PBS = Phosphate buffered saline

GF = Gingival fibroblast

CT group = Control groups

COM group = Compression groups

Vi groups = Vibration groups

CV groups = Compressive combined with vibraion groups

Real time-PCR = RNA isolation and Real time polymerase chain reaction

RNA = Total ribonucleic acid

mRNA = Messenger ribonucleic acid

GAPDH = Glyceraldehyde-3-phosphate dehydrogenase

ELISA = Enzyme-linked immunosorbent assay

CHAPTER 1

INTRODUCTION

Background and rationale

Orthodontic tooth movement induced by applied mechanical force depends on the remodeling process of periodontal tissue, dental pulp, alveolar bone, periodontal ligament (PDL), and gingiva [1]. When teeth are affected, the movement will immediately occur within tooth socket. On the side of alveolar bone that is the same direction of tooth movement is the compression side, which the bone resorption will follow in the future. On the other side, PDL fibers will be stretched and called "tension side" which bone formation will occur. That means, PDL distributing the force exerted on the teeth [2]. Moreover, studies indicate that PDL cells are highly sensitive and response to mechanical stimuli that play the major role in initiating PDL and alveolar bone remodeling process during orthodontic tooth movement [2, 3]. Furthermore, majority of cells in PDL will be stimulated and mediated largely through fibroblasts according to the research that fibroblasts are considered to be mechanoresponsive cells [2, 4, 5]. The PDL fibroblasts response to mechanical stimuli by secreting of various molecules, for example, cytokines, colony-stimulating factors, growth factors (GFs) and inflammatory mediators. These molecules will stimulate the response of cell in each cell around tooth roots and create proper microenvironment for PDL and alveolar bone remodeling process [5, 6].

The applied orthodontic force will affect PDL vascular system that will cause PDL's capillaries obstruction and consequently ischemia and hypoxia, which shall maintain cells in PDL. As the result, sterile necrosis of cells shall take place in hyalinized area feature that restrain tooth movement. To resume the tooth movement, the necrotic tissues and alveolar bone adjacent compressed PDL area has to be eliminated [1]. Clearly, bone resorption in compression side of PDL is essential for the tooth movement. Therefore, the rate of bone resorption in compression side of PDL determines the rate of tooth movement [7]. Bone resorption can occurs via induction of osteoclasts or osteoclastogenesis, which consists of differentiation of osteoclastic precursors into mature osteoclasts and activation of mature osteoclasts. There are two factors influence to the formation of mature osteoclasts. The first is receptor activator of NF-*k*B ligand (RANKL) and osteoprotegerin (OPG). The second factor is macrophage colony-stimulating factor (M-CSF), which is necessary for the differentiation and proliferation of osteoclast precursors. However, there are several types of cytokines that affect osteoclastogenesis process, bone resorption and production of RANKL such as interleukin-1β (IL-1β) and tumor necrosis factor - α (TNF-α) [7, 8]. Combinations of cytokines, such as M-CSF, IL-1β and TNF-α, induced bone marrow cells to differentiate into bone resorbing osteoclasts and osteoclast proliferation [9]. It is found that pressure from orthodontics that stimulates fibroblasts to release IL-1β, TNF-α [10] and also found that IL-1β, TNF- α will be pro-inflammatory cytokines that is highly active in compression side comparing to tension side during early stages of orthodontic tooth movement [11, 12]. The study indicates the role of IL-1β and TNF-α on tooth movement that both of them react to osteoclastic activity and survival while stimulate osteoblasts to create chemokines CCL-2, 3, 5 in order to induce osteoclast precursors into bone resorption area [10]. In addition, it is also found that they cause RANKL expression to react with RANK on osteoclasts precursors, which initiates osteoclastogenesis [13]. Overall, it can be concluded that both IL-1β and TNF-α are significant in bone resorption. They affect osteoclastogenesis by RANKL and OPG stimulation in osteoblasts and also directly affect osteoclasts and their precursors. Above-mentioned, the majority of rate in tooth movement depends on rate of bone resorption in compression side. Therefore, to accelerate tooth movement, bone resorption process shall be accelerated by increase activation of IL-1β and TNF-α.

The acceleration of tooth movement has long been required for its multiple usefuls, including reduce orthodontics period in order to prevent side effects such as gingival recession, periodontal disease and root resorption which may occur after a long orthodontics period [7]. However, it is still a challenge to accelerate tooth movement. There were many methods to accelerate tooth movement and try to develop method from invasive to less invasive methods as follow: biological approaches by injecting exogenous inflammatory mediators and hormones for bone resorption such as prostaglandin E (PGE_1 and PGE_2) [14], Vitamin D [15] and PTH [16], local RANKL gene transfer to periodontal tissue [17]. But all of these agents have some or the other unwanted adverse effects such as root resorption and pain. Thus, there is no any mediator or hormone can accelerate orthodontic tooth movement without the side effect. After that the surgical method had been used to accelerate tooth movement such as: interseptal alveolar surgery [18], osteotomy and corticotomy [19] and piezocision technique [20]. From the principle of these methods, the inflammation cascade will begin after the bone was irritated surgically which cause faster tooth movement due to the increase in osteoclastogenesis. However, these methods are invasive and not well accepted by the patients. Therefore, non-invasive methods, mechanical or physical approaches, have been widely used. These approaches include direct electric currents [21], static magnetic field and pulsed electromagnetic field [22] or low-level laser irradiation [23]. Even though they were able to accelerate tooth movement but there were also side effects such as thermal damage to dental pulp [24].

Currently, resonance vibration with low magnitude high frequency is introduced to stimulate tooth movement, as its production is available in the market. However, the study of tooth movement is yet to be clarified. Furthermore, there are controversial of the effect of vibration to tooth movement, some studies said it restrained tooth movement, some said it accelerated tooth movement. For example, from the study of Nishimura M et al. 2008 [25] reported that vibration in combination with orthodontic force in rats could increase the velocity of tooth movement and found the expression of RANKL, the number and the resorptive activities of osteoclasts in PDL on the compression side were increased. In addition to the study of Kau et al. 2010 [26] which reported short durations of low-magnitude, high- frequency resonance vibration (30 Hz, 20 g (0.2 N) for 20 minutes combined with orthodontic force. It was shown that the rate of orthodontic tooth movement was increased without additional tissue damage in humans. In addition, the study of Leethanakul et al. 2015 [27] using the electric toothbrush with a rotating and vibrating (125 Hz, 5 minnutes, 3 times/day) with compression accelerated the canine movement by enhancing of the IL-1β levels in GCF and appeared to increase bone resorption activity with no pathological effects after application of such vibratory stimuli to a single tooth for a short period of time. All three studies demonstrated the acceleration of tooth movement by vibration. Nevertheless, there is also a contrary study indicating that vibration does, indeed, restrain tooth movement. In vivo studies found that vibration reduces the rate of tooth movement because it affects alignment of the periodontal ligament fibers in rats, inhibit osteoclastogenesis and bone resorption, in addition to the reduction of osteoclast formation. Besides, there is increase in markers of osteoclastogenesis after LMHF mechanical vibration stimulation [28, 29]. However, the commercial device of vibration is available even the result is controversy including the unclear mechanisms to accelerate the tooth movement has not been investigated. Therefore, the signal transduction pathways of vibration to tooth movement and the consequence need to be studied including the response of periodontium. This is the reason why the effect of LMHF mechanical vibration during orthodontic force is importance to study whether it will accelerate tooth movement or not. If so, it shall be a relevance of bone resorption's stimulation which according to the rate of majority of the tooth movement depends on the rate of bone resorption in compression side and pro-inflammatory cytokines that strongly effect to bone resorption are IL-1β and TNF-α [10, 30]. Therefore, if the tooth movement is accelerated, the process of bone resorption will be activated extremely that means the level of IL-1β and TNF-α should be increased significantly.

This study was designed to study the effects of LMHF mechanical vibration on the acceleration of orthodontic tooth movement in the compression side of PDL via the expression of IL-1β and TNF-α at the genetic levels of PDL fibroblasts. The molecular level of these mechanisms may provide the clinicians to understand how the LMHF mechanical vibration accelerates orthodontic tooth movement and may be the basic knowledge that supports the use of a vibratory stimulation device to shorten the orthodontic treatment time.

Review of Literature

Phases of orthodontic tooth movement

Based on the study of Burstone CJ. 1962, the phase of orthodontic tooth movement is divided into three phases: the initial phase, lag phase and post-lag phase. While as, recent studies divide the phase of orthodontic tooth movement into four phases, the first phase takes 24-48 hours and showed the movement of tooth within bony socket. Simultaneously, the second phase which the teeth stops movement about 20-30 days because of the presence of hyalinization or necrotic tissues. When the necrotic tissue is removed, the movement of teeth is turn to accelerate that called "the third phase" and the movement of teeth continues into the fourth phase. Both phases is the phase which with the most of the overall tooth movement. However, many studies showed that the third and fourth phases as same as a post-lag phase in Burstone. In conclusion, orthodontic tooth movement can explain into 3 phases of orthodontic tooth movement.

After force application, the initial phase of tooth movement will start immediately. Because PDL fibers and cells were compressed and stretch in pressure and tension side of PDL, respectively, acute inflammatory process starts. The presence of hyalinization in the pressure side was shown in this early stage.

Form the second phase, compression areas are quickly detected by the distortion of the PDL fiber arrangement. The disruption and occlusion in capillaries leads to hyalinized formation and restrain of tooth movement, which can take approximately about 4 to 20 days. The movement of tooth allows to resume when necrotic tissue is removed, including bone resorption near bone marrow spaces and near the direction of the feasible PDL.

In addition, the acceleration and linear phases are the third and fourth phases of tooth movement are, respectively, started on 40 days after the force applied [1].

Theories of orthodontic mechanisms

There are two major mechanisms for tooth movement as follows

1. .The pressure-tension Theory

2. The bone-bending theory

The pressure-tension Theory[1]

This classic theory hypothesized by Sanstedt (1904), Oppenheim (1911), and Schwartz (1932), explained that the movement of tooth within PDL space create a "pressure side" and a "tension side". In addition, Schwarz hypothesized that PDL is a continuous hydrostatic system. When forces is applied then hydrostatic pressure would be create on PDL. According to Pascal's law, hydrostatic pressure will be diffuesd equally to all regions of PDL. On the pressure side occur vascular constriction leading to decrease in cell replication and causing bone resorption. On the opposite side "tension side", PDL fibers are stretched, so, cell replication will be increasing and causing bone formation.

The bone-bending theory[1]

This hypothesis was introduced by Farrar then confirmed by Baumrind in rats and Grimm in humans, believed that bending of alveolar bone plays key role in orthodontic tooth movement. When applied forces, forces are transmitted to tissue around tooth. These forces cause bending of bone, tooth and hard structure of PDL. In addition, elasticity of bone more than others tissue, so, bone will be bent over to response applied forces [31].

The force applied to the tooth is scattered to the bone by increasing of stress lines and another force application develop into a stimuli for changed the response of cells lying perpendicular to the stress lines. The bone bending followed by active biologic processes that involved bone remodeling of cellular and inorganic portions. All of this processes are increased while the bone is controlled in the deformed position. External forces makes the changed of cell activities turn to reshape of cells and internal organization of bone and then bends the bone.[31]. It has been recommended by Davidovitch et al. 1980 that mechanical and electrical perturbation of bone exist a physical relationship. Electrical effects generation by stress due to bending of bone. Their studies with exogenous electrical currents in combined with orthodontic forces determined enlarged activity of cells in the PDL and alveolar bone, further accelerated tooth movement [32, 33]. Taken together, these findings suggest that the response of bioelectric current generated by bends of the bone circumstance to applied orthodontic forces might function as crucial the first messengers of cell.

Orthodontic tooth movement

The orthodontic tooth movement exerts physical, biophysical and biochemical effects on extracellular matrix (ECM) and constituent cells of the periodontium and dental pulp [1, 4]. Immediately after force applied, the tooth moves within its socket. This movement cause strain in ECM of the PDL and elicits fluid flow in the PDL and alveolar bone. Together, according to the fluid flow theories, a strain in bone activates fluid flow in canaliculi, which cause a shear stress on osteocytes and microfrature of the bone [4]. Besides, strain and fluid flow within the PDL lead to PDL cells deformation especially PDL fibroblasts and alters PDL's microenvironment due to PDL's vascularity alterations and blood flow producing local synthesis and release of different inflammatory cytokines and mediators[4, 34].

After the cells in PDL and bone are activated, these cells responses by production of mediators such as neurotransmitters (e.g., vasoactive intestinal polypeptide, calcitonin gene-related peptide (CGRP) and substance P), arachidonic acid metabolites (e.g., PGE), cytokines (e.g., tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 8 (IL-8), receptor activator of nuclear factor kappa B ligand (RANKL) and gamma interferon (IFN-γ), growth factors (e.g., fibroblast growth factor (FGF) , transforming growth factor β (TGFβ), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and connective tissue growth factor (CTGF)), and colony stimulating factors (e.g., granulocytes colony stimulating factors (G-CSF), granulocytes macrophages colony stimulating factors (GM-CSF) and macrophages colony stimulating factors (M-CSF)). These molecules initiate a signals cascade that cause the stimulation of many cellular responses by different cell types within the tooth sockets contributing a proper microenvironment for bone formation or resorption [1, 35, 36].

Mediators from activated osteocytes effect on the PDL cells which differentiate into osteoblasts through releasing factor from activated osteocytes such as platelet-derived growth factor and bone morphogenic proteins (2, 6 and 9) that also activate osteoblast activity [34]. Moreover, osteoclast precursors activation and differentiation into osteoclasts are stimulated by factor from PDL cells (PDL fibroblasts and osteoblasts). At the resorption side, In vitro and in vivo studies shows soluble factor such as, receptor activator of nuclear factor kappa β ligand (RANKL), osteoprotegerin (OPG) and colony-stimulating factor regulate osteoclast differentiation [10, 37]. Before actual bone resorption can show up, osteoblasts must resorp the non-mineralized layer of the osteoid then the differentiated osteoclasts can adhere to the surface of bone [38]. According to in vitro studies, PDL fibroblasts and osteoblasts responses to mechanical force by inflammatory mediator production, such as prostaglandins and enzyme such as MMPs and cathepsins that activate degradation of ECM [39, 40]. At the formation side, Bone formation of the tooth consists of combination of ECM synthesis and mineralization. The activated produces many inflammatory mediators while bone cells stimulate ECM synthesis. For examples, members of the transforming growth factor-β (TGF-β) superfamily, cathepsin B and L were also found in the gingival crevicular fluid of the orthodontically moved teeth in humans and at the formation side of rat teeth after force applied [39, 41].

In conclusion, the complex regulatory network that stimulates PDL and bone remodeling during orthodontic tooth movement consists of fibroblasts, osteoblasts, osteocytes, and osteoclasts [34].

Periodontal ligament and cells in periodontal ligament

Orthodontic tooth movement stimulated by applied mechanical force is depends on remodeling in periodontal tissue, including dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva [1]. When tooth are affected, the movement will immediately occur within tooth socket. On one side, compression side of alveolar bone and PDL takes place and bone resorption will follow in the future. On the other side, PDL fibers will be stretched and called “tension side” which shall have bone formation. So, the application of force to the tooth is conveyed to the alveolar bone over the periodontal ligament (PDL). PDL is a highly vascular and cellular connective tissue that connects the teeth to the alveolar bone proper, contribute support, attachment, and sensory function [5]. PDL is a specialized matrix rich, mixed cellular/dense fibrous connective tissue [36]. PDL contains several cells populations comprising osteoblasts and osteoclasts on the bone side, whereas periodontal ligament fibroblasts, undifferentiated mesenchymal cells, macrophages, neural elements, endothelial cells, smooth muscle cells, and epithelial cell rests of Malassez in the body of the PDL, in addition cementoblasts on the root surface. All of these cells, the majority cell type is the periodontal ligament fibroblasts. In this study, only PDL fibroblasts are referred to as PDL cells. PDL cells also play a major role in alveolar bone remodeling process during orthodontic tooth movement as well as osteoblasts and osteoclasts [4]. One of the functions of PDL cells is to resist and respond to the forces provoked by mastication, speech and orthodontic treatment. Commonly, because of a lack of PDL, ankylosed teeth cannot be moved by mechanical force [42]. There are evidences that in the orthodontic tooth movement model, strains in the alveolar bone are generally lower than 0.02% [43, 44] which not sufficient to stimulate bone cells to generate the bone remodeling process [45]. On the contrary, strains which generate in the PDL have been found to be in the order of 10–40% [43, 44] which above the threshold that required to stimulate fibroblasts to initiate the remodeling process (strain levels around 7–12%) [46-48]. Above-mentioned, strains in PDL and the response of PDL cells to mechanical stimulus play the improtant role in initiating remodeling process during orthodontic tooth movement [2, 43, 44]. PDL cells response to mechanical stimuli by form biologically active substances, such as cytokines and enzymes for signaling the surrounding cells to control the bone matrix resorption and formation [2, 5]. Furthermore, several studies have determined that mechanical stress can also effect the expression and secretion of several cytokines and proteinases by PDL cells [49--58].

Tissue and cell changes during orthodontic tooth movement:

Compression side

The compression side is an area that is compressed by applied orthodontic forces in the same force direction. Compression lead to the PDL's capillaries deformation and disarrangement of tissues around the teeth. After that, blood flow and periodontal tissue alters may modify to the compression force. The changes of metabolite can develop to the PDL cells because of hypoxia and decreased nutrient levels [1]. In hypoxic conditions, cells will rely on anaerobic glycolysis. Many enzymes involved in an anaerobic metabolism can be potential markers. For example, Lactate dehydrogenase is a molecule that accumulates during anaerobic metabolism. Cells that adapt via metabolic changes will continue to live and cells that cannot adapt to the ischemic condition will die [59]. The dead cell will break up, releasing all of its contents to the environment and subsequently causing the activation of local inflammatory processes, featured by vasodilation and migration of leukocytes out of capillaries [1].

Local hypoxia induces the PDL fibroblasts increasing the PGE_2 expression, pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) and VEGF [60]. Subsequently, IL-1β and

TNF-α stimulate up-regulation adhesion molecule expression (VCAM-1 and ICAM-1) in endothelial cells which in order to enhance leukocyte adhesion and migration [61]. In addition, when nerve fiber was compressed by mechanical stimuli, then its released vasoactive neurotransmitters such as CGRP and Substance P [1, 35]. At the time, CGRP, PGE_2, and VEGF together with in vasodilation of inflammatory process, leading to increase blood flow and promote leukocyte migrate from capillaries to inflammatory area [35]. Then, the recruited leukocytes coordinate directly or indirectly with the entire population of native paradental cells to increase specific chemokines production, GFs and cytokines that involved in bone resorption process.

Furthermore, osteocytes,which induced by fluid-flow and hypoxia, releaseed HIF-α [61] and produce signaling molecules to osteoblasts. Then, osteoblasts release chemokines MCP-1 (CCL2) to induce osteoclast precursors migrate to bone resorptive area. Moreover, both IL-1β and TNF-α from PDL fibroblasts can activate osteoblasts to release chemokines such as CCL2, CCL3, CCL5 taken together CXCL12 from Bone vascular endothelials cells and marrow stromal cells to induce osteoclast precursors migrate to bone resorptive area, too [10].

Bone resorption occurs via osteoclasts function or osteoclastogenesis. There are two factors that regulate osteoclastogenesis, the first is RANKL/OPG and second is M-CSF [62]. As same in the study have been shown that the RANK/RANKL/OPG axis play important roles in osteoclastogenesis [36]. These processes occurs when RANKL and M-CSF binding with RANK and c-Fms receptor on osteoclasts precursors, respectively. After that, osteoclast precursors differentiate to mature osteoclasts and bone resorption will occurs [35, 63]. Nevertheless, this interaction between RANKL and RANK could be restrained by osteoprotegerin (OPG), which is created by osteoblasts. OPG acts as decoy receptor for RANKL following by the restraint of bone resorption [35]. (Figure 1)

Osteoclastogenesis, regulation of osteoclastogenesis and bone resorption

The osteoclast is a primary bone-resorbing cell, which originate from mononuclear precursors and fuse to form multinucleated osteoclasts. The more nuclei in the degree of multinucleation cause more bone resorption. Both systemic hormones and cytokines released locally in the microenvironment of bone regulate osteoclasts differentiation and activity. Furthermore, another cell in the bone marrow can effects osteoclasts activity and formation. These cells consist of T and B-lymphocytes, marrow stromal cells, osteocytes, and osteoblasts. All of cell types release chemokines and cytokines which activate or inhibit osteoclastogenesis[64]. The pluripotent hematopoietic stem cell is differentiated to osteoclast and causes a myeloid stem cell, which can another differentiate to megakaryocytes, granulocytes, monocyte-macrophages, and osteoclasts. The granulocyte-macrophage colony-forming cell (CFU- GM) is the earliest detectable hematopoietic precursor that can differentiate to osteoclast. Early osteoclasts precursors are proliferative cells, which can expand in numbers to response to hematopoietic growth factors such as IL-3, GM-colony-stimulating factor (CSF), and M-CSF [65]. Moreover, M-CSF block apoptosis of the precursors. The early osteoclasts precursors differentiate and proliferate to form a post-mitotic committed osteoclast precursor (Figure 2). These authorized osteoclast precursors under the effect of RANK ligand (RANKL) or 1, 25-$(OH)_2D_3$ then differentiated and fused to form immature osteoclasts and followed by stimulated to form active osteoclasts. RANKL, IL-1β and TNF-α are the factors that can stimulate osteoclast [66].

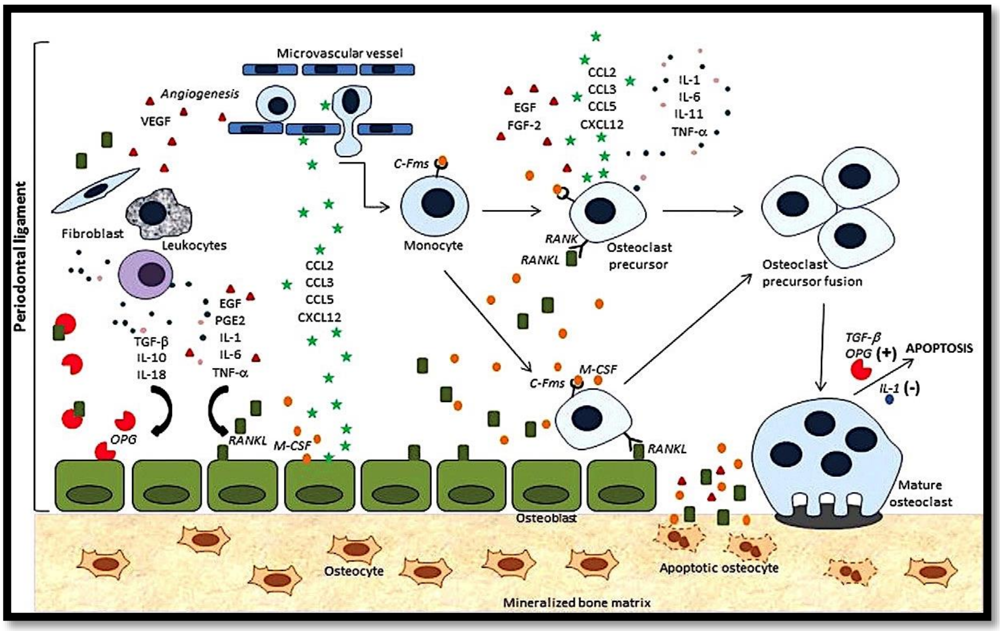


Figure 1: Shows tissue and cellular response to orthodontic force in compression side of PDL**.**

Currently, it is accepted that the systems allowed for creation of two haematopoietic factors which are both needed and sufficient for osteoclastogenesis, RANKL and M-CSF, and for the consequent stimulation of RANK on the haematopoietic precursor cells surface [35, 63]. Taken together, M-CSF and RANKL are essential to activate gene expression that illustrate the lineage of osteoclasts, including those encoding tartrate-resistant acid phosphatase (TRAP), cathepsin K (CATK), calcitonin receptor and the β3-integrin, causing the mature osteoclasts formation. The activated osteoclast survival for 2 weeks in the bone marrow and followed by apoptotic process. Factors that can increase osteoclast apoptotic osteoclasts include bisphosphonates and TGF-β [58].

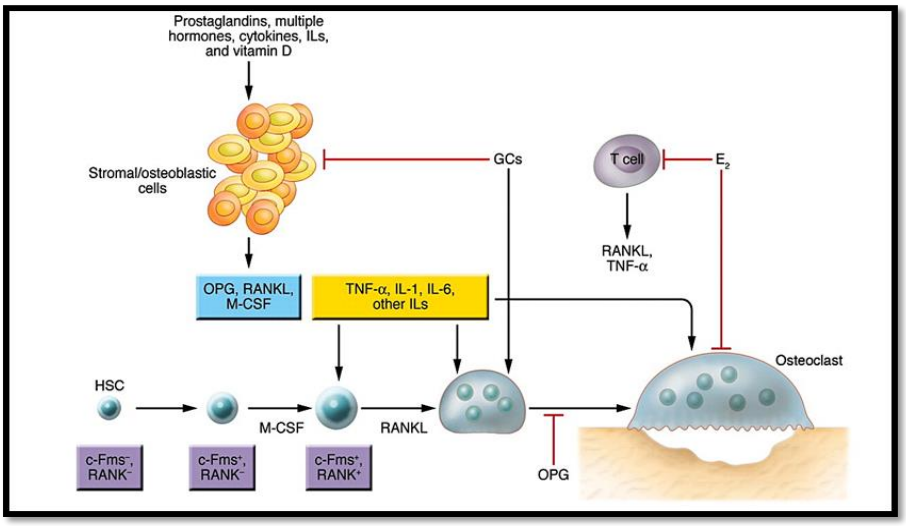


Figure 2: shows Life cycle's osteoclast from proliferation and differentiation to apoptosis

Remodeling is controlledthe of RANKL amplified surface of cells support stimulation bone resorption and When the concentration of OPGs are highly relative to RANKL expression, OPG binds to RANKL, inhibiting it from binding to RANK. Formation of osteoclasts and apoptosis of pre-existing can scale down by impeding the binding of RANKL to RANK [35]. In addition, pro-inflammatory cytokines, such as IL-1β, IL-6, IL-11, and IL-17 and TNF-α, can stimulate osteoclastogenesis by increasing RANKL expression whereas decreasing OPG production in osteoblasts/stromal cells. On the other hand, anti-inflammatory mediators, such as IL-13 and IFN-γ, may cut down the expression of RANKL and/or increase OPG expression to block osteoclastogenesis [10, 36]. (Figure 3 and Figure 4)

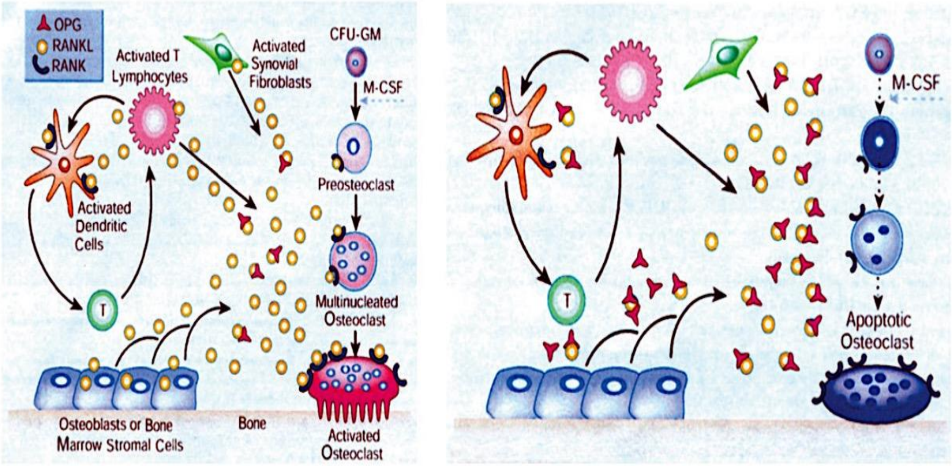
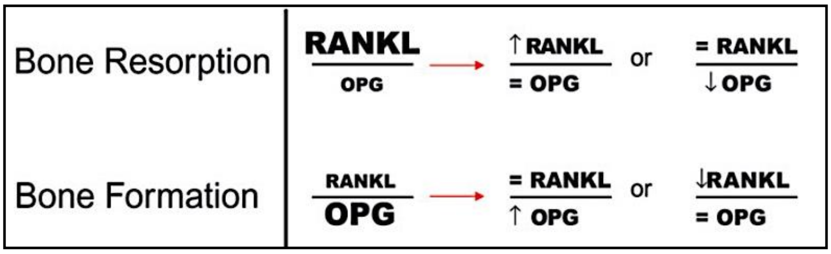


Figure 3: shows mechanism of action of RANKL expression by various cell types in the induction of osteoclastogenesis following binding to RANK on osteoclast precursors (left). An abundance of OPG relative to RANKL (right) inhibits RANKL binding to RANK, bring about reduced osteoclastogenesis and the promotion of apoptosis of existing osteoclasts [35].



Bone Resorption	$\frac{\textbf{RANKL}}{\text{OPG}}$	→	$\frac{\uparrow \text{RANKL}}{= \text{OPG}}$	or	$\frac{= \text{RANKL}}{\downarrow \text{OPG}}$
Bone Formation	$\frac{\text{RANKL}}{\textbf{OPG}}$	→	$\frac{= \text{RANKL}}{\uparrow \text{OPG}}$	or	$\frac{\downarrow \text{RANKL}}{= \text{OPG}}$

Figure 4: Even if bone remodeling occurs depends desperately on the RANKL/OPG ratio, which is a relative expression level function of RANKL and OPG [35, 67].

Role of Periodontal ligament fibroblasts in osteoclastogenesis

Periodontal ligament fibroblasts (PDLFs) are spindled-shaped and elongated connective tissue cells that are placed in the PDL. Particularly feature of human PDLFs and osteoclast progenitor’s cells may cooperate in the process of differentiation of osteoclasts. When co-culturing human PDLFs with osteoclast precursors found that human PDLFs will express adhesion molecules such as ICAM-1 to attract and select osteoclast precursors. Then, human PDL up-regulation of osteoclastogenesis-stimulatory molecules such as macrophage-colony stimulating factor (M-CSF), tumor necrosis factor-α (TNF-α) and receptor activator of nuclear factor-κB ligand (RANKL) and osteoclast precursors express TRAP followed by osteoclasts precursors migrate to the surface of bone. The function of human PDLFs in osteoclasts formation is promotors. (Figure 5)

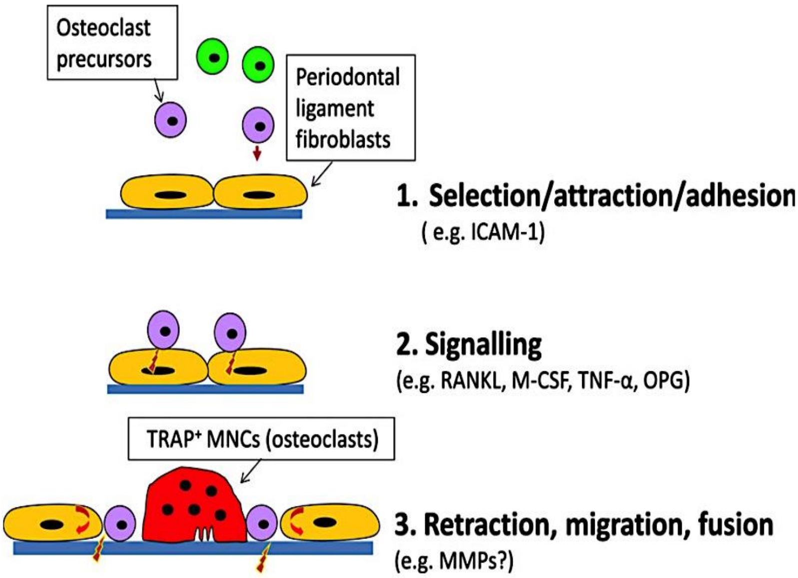


Figure 5: Periodontal ligament fibroblast (PDLF)-regulated osteoclast formation process [66] .

Interleukin-1beta (IL-1β)

IL-1, pro-inflammatory cytokine, is directly elaborated in bone resorption process by joining in the survival, fusion and activation of osteoclasts. In addition, There are 2 forms of IL-1: IL-1α and IL-1β which activities by binding to the receptors, IL-1-RI and IL-1-RII [68]. Furthermore, IL-1 can stimulated by various stimuli such as neurotransmitters, production of bacterias, other cytokines, and mechanical stress. The action that mentioned-above includes captivating leukocytes and activating fibroblasts, endothelial cells, osteoclasts, and osteoblasts to enhance bone resorption and inhibit bone formation [1]. The target cells for IL-1 is Osteoblasts, which in transfer messages to osteoclasts for bone resorption. However, between IL-1α and IL-1β, IL-

1β is a dominant physiologic form of IL-1 and seem to be more potential, moreover, mainly involved in bone metabolism [69, 70], including stimulate bone resorption and inhibit bone formation [71].

In addition, IL-1β plays a crucial role in the inflammatory process that secreted by monocytes, macrophages, fibroblasts, endothelial cells, and epidermal cells and its secretion is also stimulated by various stimuli. According to the studies have been shown that the level of IL-1β in GCF during orthodontic tooth movement was elevated significantly in the first 7 days of tooth movement and highest at 24 hours and then diminished to baseline level during the linear phase of tooth movement [13, 72, 73]. Furthermore, when received to continuous forces (0.5-3.0 g/cm^2) or intermittent forces (2.0 or 5.0 g/cm^2) mechanical compressive stress, PDL cells stimulate osteoclastogenesis in vitro by down-regulation of the expression of OPG and up-regulation of the expression of RANKL, via PGE_2 and IL-1β synthesis [49, 74]. In addition, IL-1β can stimulate the formation of osteoclasts directly from osteoclast precursors under TNF-α stimulation, in vitro study [75]. Based on the study about the rate of tooth movement found the positive correlation ratio of IL-1β and IL-1 receptor antagonist [76].

Tumor necrosis factor – alpha (TNF-α)

TNF-α is a multifunctional pro-inflammatory cytokine that released primarily by activated monocytes and macrophages, but also by osteoblasts, epithelial cells, fibroblasts and endothelial cells [77]. TNF-α is one of the most dominant osteoclastogenic cytokines produced in inflammatory process and also important factor for the differentiation of osteoclasts [78]. So, TNF-α mediates RANKL activation of osteoclast differentiation through an autocrine mechanism [79]. Determined as a dominant bone resorption factor, TNF-α is important for activating osteoclastic bone resorption in vitro [80]. This cytokine applys its osteoclastogenic effect by stimulating NF-κB through an intracellular mechanism overlapping those of RANKL. In addition, when TNF-α binds with its p55 receptor on surface of osteoclast precursors, then they stimulates directly and indirectly osteoclastogenesis process and up-regulating the expression of RANKL, M-CSF, and other chemokines on osteoblasts. TNF-α is also an apoptotic factor for osteocytes, which could be the noticeable for the recruitment of osteoclast to resorb bone in the PDL pressure side, meanwhile inhibiting osteoblasts [78].

In bone resorption, the exactly role of TNF-α are up-regulate and gain the amount of orthodontic tooth movement which was found in rodent models with the impairment of TNF-α receptor. As same in vitro study showed that, in compression side, PDL fibroblasts release greater amount of TNF-α than at the tension side in PDL [11]. Simultaneously, local TNF-α treatment or activation of cells that produce this pro-inflammatory protein might be a future alternative to faster orthodontic tooth movement. In addition, injection of local TNF-α antibody might be effective method to enhance the anchorage region during OTM [78].

Methods for accelerate orthodontic tooth movement

Accelerating movement of the teeth has long been required for its multiple useful, such as reduced treatment duration, promoted envelope of tooth movement, reduced side effect (Examples: oral hygiene related problems, root resorption, and gingival recession), improved post-treatment stability and differential tooth movement [81]. Currently, accelerating movement of the teeth is still challenging. An amount of methods have been designed to make various approaches in order to achieve faster results, but still have many side effects and uncertainties results toward most of all the methods. Most methods can be classified into biological, physical, biochemical, and surgical approaches [82].

Biological approach

The movement of teeth stimulated by the application of mechanical forces followed by remodeling of alveolar bone and periodontal ligament (PDL) process. Bone remodeling consists of two processes which are bone resorption at the compression side and bone formation at the tension side. The application of forces on the teeth will lead to alter in the microenvironment within the PDL due to alterations of blood flow, causing the excretion of various inflammatory mediators such as colony-stimulating factors, growth factors, neurotransmitters,, arachidonic acid metabolites and cytokines. Subsequently of these mediators, bone remodeling process occurs [1].

There are experiments that using these molecules exogenously to promote tooth movement such as prostaglandin E (PGE_2), cytokines such as IL-1β, RANKL, and M-CSF. Prostaglandins (PGs) are inflammatory mediator and a paracrine hormone that activates resorption of the bone by increasing directly osteoclast numbers. In vivo and in vitro studies have been found that PGs, applied forces, and the acceleration of tooth movement have the positive relationship. In addition, experiments have shown that exogenous PGE_2 injections for a long time generated faster tooth movements [83]. However, the different concentrations and number of injections can effect and relate to resorption of the root and pain [84].

Vitamin D3 has been widely appeal to many researchers because its role in the faster tooth movement; 1, 25 dihydroxycholecalciferol (hormonal form of vitamin D) plays a key role in homeostasis of calcium with calcitonin and parathyroid hormone (PTH) [82]. On the two groups of rats which compared between local PGE_2 and exogenous of vitamin D injections, showed that there is no significantly in acceleration between the two groups. Both PGE_2 and Vitamin D promoted the amount of tooth movement showed that experimental group was significantly greater than the control group. The Howship's lacunae numbers and capillaries on the pressure site were significantly higher in the PGE_2 group than in the vitamin D group. On the other hand, the osteoblasts number on the external surface of the alveolar bone on the pressure site was significantly higher in the vitamin D group than in the PGE_2 group. Therefore, vitamin D was found to be more powerful in inflecting bone turnover during orthodontic tooth movement due to its effects on bone resorption and bone formation were well balanced [85].

Parathyroid hormone (PTH) is one of the effective stimulatory osteoclast formation factors. There is report established that parathyroidectomy completely inhibited formation of osteoclast at the compression side of periodontal tissue in experimental tooth movement and resumption occurred after injection of extracted parathyroid. Therefore, PTH plays a key role in osteoclast formation on the compression side of periodontal tissue by mechanical stimuli. In vivo investigated affect of parathyroid hormone on experimental tooth movement in rats showed that tooth movement and osteoclast numbers were significantly increased in the parathyroid hormone group compared with the control group. The expressions of receptor activator of nuclear factor kappa B ligand and insulin-like growth factor-I were significantly stimulated in the parathyroid hormone group. In conclusion, short-term parathyroid hormone injection might be a potential method for accelerating orthodontic tooth movement by increasing the alveolar bone turnover rate [86].

RANKL, membrane-bound protein on the osteoblasts, bind to the RANK (osteoclasts surface) then leads to osteoclastogenesis [87, 88]. In contrast, OPG is decoy receptor that competes with RANKL to bind with osteoclast and block osteoclastogenesis. The RANKL-RANK-

OPG system plays a key role in balance process of bone remodeling. According to this, using these biological molecules (RANKL, OPG) in the faster tooth movement, In vivo showed that the up-regulation of osteoclastogenesis and faster tooth movements in rats is done by transferring RANKL gene to the periodontal tissue [17]. Although clinical trials on humans about the administration of exogenous biological molecules to reduce orthodontic treatment time has been limited but there are intensively tested on animal studies. Furthermore, Administration of biological molecules by local injections because of avoiding of systemic effects. However, local injection can be painful and patients may be discomfort including root resorption but the side effect from these methods was not investigated for long periods of time.

Physical approach

Physical approach in stimulating tooth movement is by using device-assisted treatment including pulsed electromagnetic field, direct electric currents, static magnetic field, low-level laser and resonance vibration. Physical approaches uses concept of bone bending theory and bioelectrical potential develops [82]. Zengo et al. 1974 found that the concave side of alveolar bone has negatively charged inducing osteoblasts to this area. On the opposite side, the convex side has positively charged inducing osteoclasts to this area. According to this concept, clinical research use the cyclical force device with the vibration rate was 20-30 Hz and 20 min/day in patients and received 2 to 3 mm/month of tooth movement[32]. Anyway further results needed to be studied to certainly identify the optimal range of Hertz which can be used in these researches to get the maximum required results.

Currently, another approach, which one of the most approaches, is to use low-level laser therapy or photobiomodulation. Low-level laser has a biostimulatory effect on bone regeneration, which has been found in the midpalatal suture during rapid palatal expansion, and bone resorption by activates the proliferation of osteoclasts, osteoblasts, and fibroblast, moreover, effects bone remodeling that can accelerate tooth movement. This approach will be produce ATP and activate cytochrome C, it's from low-energy laser radiation, to enhance the speed of tooth movement via RANK/RANKL and the macrophage colony-stimulating factor and its receptor expression. Furthermore, both in vivo and clinical researches have shown that low-level laser can accelerate orthodontic tooth movement. However, contradictory results has been shown related to the low-level laser therapy. Thus, more investigates are needed to studied the optimum energy, wavelength, and the optimum duration for usage.

Surgical approach

Surgical approaches consist of interseptal alveolar surgery, osteotomy, corticotomy, and Piezocision technique that have been tested in turn to accelerate tooth movement.

Interseptal alveolar surgery or distraction osteogenesis, this method will reduce the resistance on the pressure site. Liou EJ et al. 1998 used this technique by undermined the interseptal bone distal to the canine after first premolar extraction about 1 to 1.5 mm in thickness, and dig the socket by a round bur along to length of the canine. After the surgery, canine retraction is activated by an intraoral device directly. It has been demonstrated that full retraction about 6 to 7 mm of the canine to the socket after first premolars extration took 3 weeks [89].

Osteotomy is a segment cutting into the medullary bone, after that the bone is separated and is moved as a unit. Whereas corticotomy involves only cortical bone, in the decortication form of lines and dots operated around the teeth that are to be moved. This will lower the resistance of the cortical bone and generate a state of increased tissue turnover and a transient osteopenia, which is followed by a accelerate rate of orthodontic tooth movement[90]. The reported shown that this method have postoperative stability and enhanced retention [91]. However, the results from osteotomy more investigates are still needed to be done. The side effects from these surgical techniques is invasiveness and the short period of time to accelerate tooth movement which was only in the first 3 to 4 months and it reduces with time to the same level of the controls [92, 93].

Piezocision technique is a new, minimally invasive procedure to achieve similar results rapidly and with minimal trauma [94]. Piezocision is an encouraging tooth acceleration technique because of the least invasive in the surgical approach and its different advantages on the periodontal, aesthetic, and orthodontic conditions.

The surgical method, the most tested and the most clinically used with known prognosis and stable results. Although, it is aggressive, invasive, painful, and pricely, and patients do not need surgery but this method is the only choice that is neccessary to have a good alignment and occlusion.

Vibratory stimulation and orthodontic tooth movement

Recently, mechanical vibration with low magnitude high frequency is introduced to accelerate tooth movement as its production is out in the market. However, the study of tooth movement is yet to be clarified. Moreover, the study about mechanisms of accelerate tooth movement by vibration are still unknown. Vibration has been applied with the main aim of increasing the velocity of orthodontic tooth movement by accelerating the periodontal and alveolar bone remodeling processes. Nishimura M et al. 2008 reported the additional vibration (60 Hz, 1.0 m/s^2, 8 min/week) combined with orthodontic force could increase the velocity of tooth movement in rats without damage to periodontal tissues. Moreover, they found the expression of RANKL, the number and the resorptive activities of osteoclasts in PDL on the compression site were increased [25]. Shimizu Y.1986 reserched the movement of the lateral incisor in *Macaca fusca* loaded with a vibratory mechanical stimuli. The vibration was used for 1.5 hours/day over 3 weeks. The results found 1.3-1.4 times faster tooth movement than receiving a static force [95]. Liu D 2010 found that orthodontic force plus vibration (4Hz, 20 µm displacement, 5 min/day, every 3 days, 21 days) could accelerate tooth movement in rat model [96]. Kau Chung H et al. 2010 accomplished 2-3 mm/month of tooth movement with the use of AcceleDent type I (30Hz, 0.2N, 20 min/day, 6 months) and patient acceptance and compliance was clinical significant[26]. In addition, Kau Chung H et al. 2011 reported that no statistically significant in root length change in patients who used AcceleDent type I (30Hz, 0.2N, 20 min/day, 6 mos) [97]. Furthermore, vibratory stimulation has been reported as a method of reducing pain after orthodontic appliance adjustment 98. According to previous studies [25, 27], can be summarize hypothesis of accelerate tooth movement by resonance vibration such as prevent of blood flow obstruction and hyalinization at compression site, increase in number of osteoclasts, increase expression of RANKL on the compression side and IL-1β.

Research Objectives

To investigate the expression of IL-1β and TNF-α which simulate by mechanical vibration on PDL cells in compression side of orthodontic tooth movement.

Research Hypothesis

Mechanical vibration can enhance osteoclastogenesis via up-regulation of IL-1β and TNF-α expression in human PDL fibroblasts.

CHAPTER 2

RESEARCH METHODOLOGY

Materials and Methods

Cell culture

Human PDL cells were prepared by modification of the method of Hacopian et al. [99]. Briefly, PDL cells were taken from the middle third of healthy, non-carious premolar teeth which were extracted from 6 healthy young patients (3 male and 3 female adolescents; 14 - 19 years of age) for orthodontic reasons after obtaining the patient's consent, and used according to an experimental protocol approved by the Institutional Ethics Committee Board of Faculty of Dentistry, Prince of Songkla University.

After the teeth were extracted, its were immediately soaked in culture medium, which consist of 10% fetal bovine serum (FBS), 1% Penicillin (10,000 unit/ml)-Streptomycin (10,000 µg/ml) and 1% Fungizone (AmphotericinB 250 µg/ml) then transferred to the cell culture research. The teeth were rinsed several times with phosphate-buffered saline (PBS). After that, scraping PDL tissue of the middle third of tooth root, to prevent contamination of gingival fibroblast and dental pulp, with surgical blades No. 15 then cultured in a 35 mm culture dish containing 3ml of culture medium , incubated in the presence of 5% at CO_2 37°C.

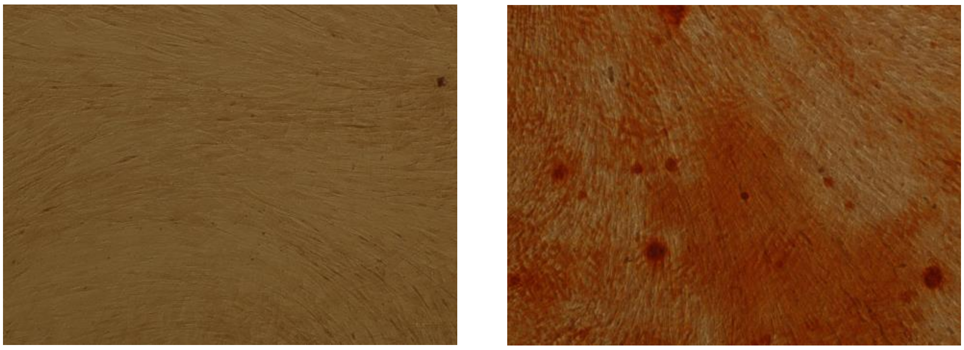
After the cells grown out of explants and reach 70%-80% confluent of cells, cells were serially subcultured by using trypsinized with 0.25% trypsin with EDTA in PBS and in 25 cm culture flasks until third to fifth passage, which was passaged in the experiment [99, 100]. PDL cells were identified by spindle-shaped cell morphology and confirmation by stains cells with Alizarin red for characterization.

PDL cells identification and characterization

All the PDL cells were identified by spindle-shaped cell morphology. The human PDL cells are sharper than human gingival fibroblast. Moreover, at confluence, human PDL cells form multilayered cultures of randomly oriented cells and growth higher rate than human gingival fibroblast, whereas human GF grow in a monolayer of parallel cells [101, 102]. In addition, there are several studies have shown that PDL cells may consist of several types of cells with unique phenotypes and distinct function. Furthermore, PDL cells can also express properties of osteoblastic phenotypes such as high alkaline phosphatase activity [102], response to parathyroid hormone, ability to produce bone-like matrix proteins and form the mineralization nodules [103].

In vitro mineralization assay

Mineralization was evaluated by stained with Alizarin red (Sigma-Aldrich, St Louis, MO, USA), following to the previous method [104]. Briefly, prepare 40 mM Alizarin red in distilled water and calibrated to pH 5.5 by using ammonium hydroxide, then applied in 12- to 24-well plates with PDL cells for 30 min at room temperature with gentle agitation. After that, the cells were rinsed by distilled water and allowed to dry before mineralization. The plate was viewed on a phase contrast microscope; human PDL cells were presented Alizarin Red-positive nodules of the calcium mineralization [103]. (Figure 6)



A. B.

Figure 6: A. PDL cells in DMEM after 21 days, B .PDL cells stained with Alizarin red.

Human PDL cells were seeded at a density of 3×10^5 cells/well in a six-well plate and incubated in DMEM with 10% FBS to promote cell attachment and grown to 70-80% confluence. Then, synchronize the cell cycle by changing the medium to DMEM with 2% FBS for 24 h. Before applied mechanical stimuli, all sample groups was changed culture medium to fresh DMEM with 10% FBS. Then randomly divided into six groups: CT groups (unloaded), COM groups (compressive stress 2 g/cm^2), Vi groups (vibration: 30 Hz and 60 Hz), and CV groups (compression & vibration).

Application of LMHF mechanical vibration

To stimulate human PDL cells with LMHF mechanical vibration, six-well plates cultured with human PDL cells seated onto a custom-made vibration platform of a GJX-5 vibration sensor (Figure 7)[29]. Put the platform parallel to the ground and then the cells were obtained LMHF perpendicular mechanical vibration. Human PDL cells in the vibrated group were received to 30 & 60 Hz of vibratory stimulation perpendicular mechanical stimuli at 0.3 g for 20 min every 24 h for 2 days. Cells, which were not received vibratory stimulation perpendicular mechanical stimuli, were cultured and sealed in a similar manner, but located on a stationary plate for the same period. After obtaining vibratory stimulation perpendicular mechanical stimuli RNA isolation and Real time polymerase chain reaction (Real time-PCR) for IL-1β and TNF-α were immediately performed.

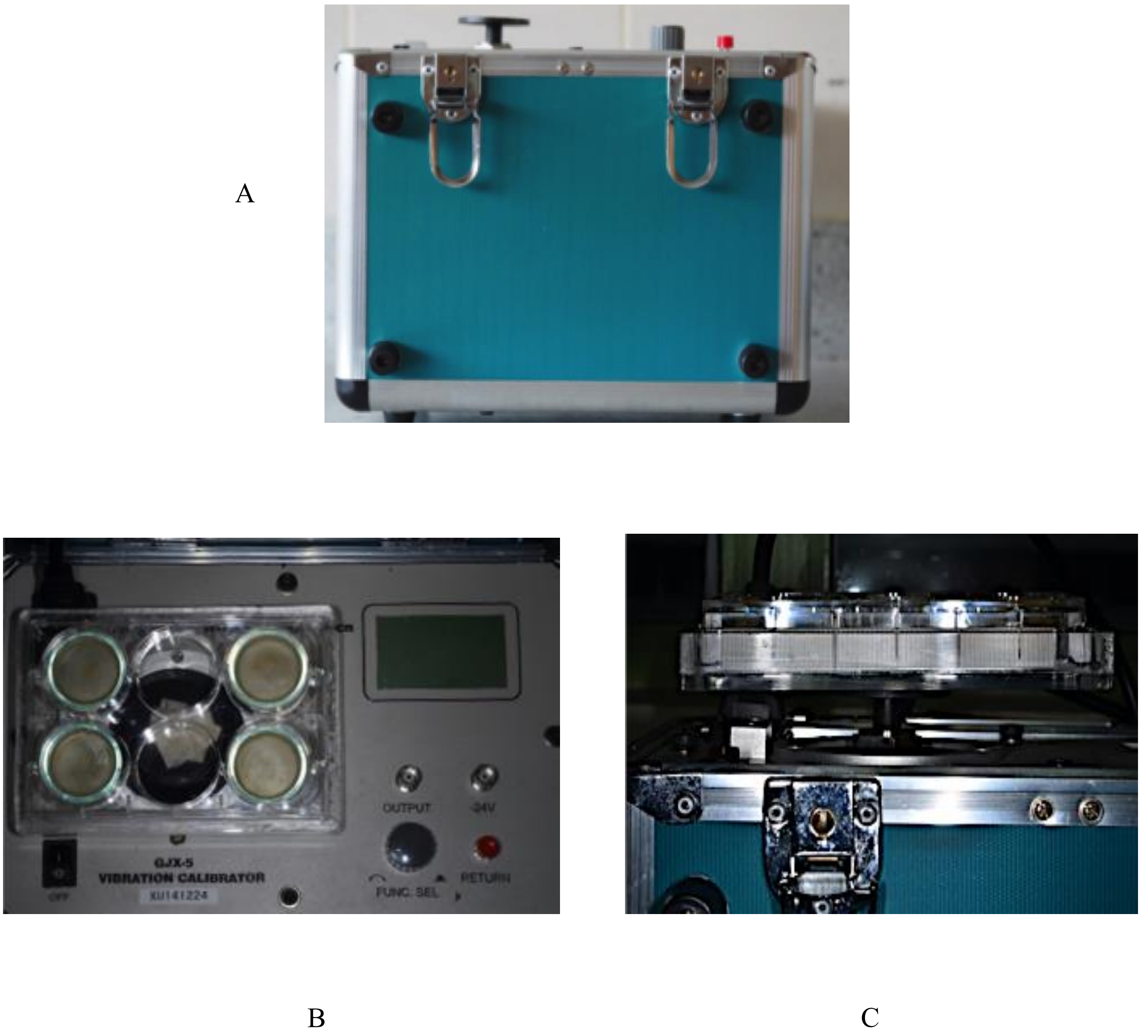


Figure 7: A. GJX-5 vibration sensor, B. & C. Human PDL cells were subjected to vibration.

Application of compressive force

In order to simulate the compression side of PDL during orthodontic tooth movement, we achieved the following *in vitro* experiment. According to a modification of the method by Kanzaki et al. [49], Human PDL cells were continuously pressed by using a custom-made compression models to stimulate the compression side of orthodontic tooth movement (Figure -8). Briefly, a 32-mm diameter base of glass cylinder was placed over 70-80% confluent cell layer in the well of a six-well plate. Compressive forces were applied by inserting the acrylic mass into the glass cylinder. The glass cylinders and the acrylic mass were washed enough with the detergent and 70% ethanol and sterilized by autoclave. Previous studies have shown that using the system utilized in the present experiment can represent compressive mechanical stress [49, 50]. The cells was received to 2.0 g/cm^2 of compressive force for 48 h.

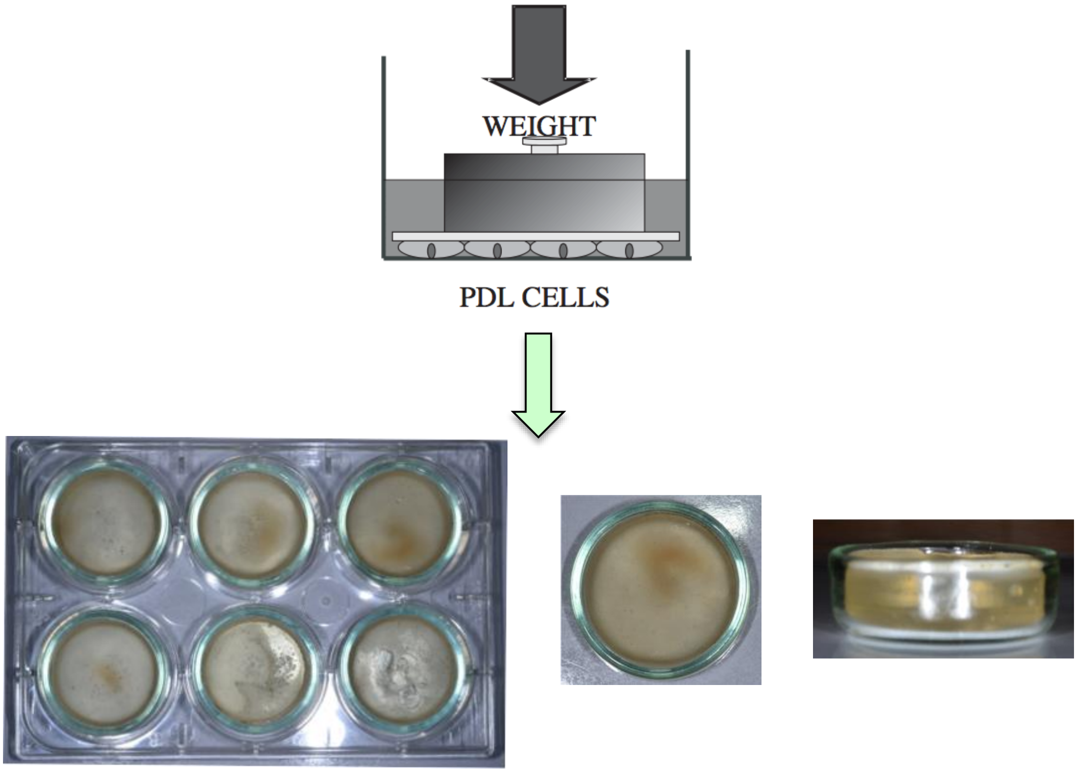


Figure 8: Compressive force model use in this study. Human PDL cells were continuously pressed by using a glass cylinder consisting of acrylic mass that generates the compressive force at 2.0 g/cm^2. The glass cylinder was placed over 70-80% confluent cell layer in the 35 mm. culture dish.

Experimental design

The expression of IL-1β and TNF-α under LMHF mechanical vibration combined with compressive stress in human PDL cells was investigated by using the frequency 30 & 60 Hzβα. Human PDL fibroblast cells were seeded at a density of 1x10^5 cells/well in a six-well plate and incubated in DMEM with 10% FBS to promote cell attachment and grown up to 70-80% confluences. Then, the medium was changed to DMEM with 2% FBS for 24 h in order to synchronize the cell cycle. Before applied mechanical stimuli, all sample groups was changed culture medium to fresh DMEM with 10% FBS. Then were randomly divided into

1. Human PDL cells that were not obtained to mechanical stimuli (Control groups)
2. Human PDL cells that were obtained to continuous compressive forces (2 g/cm^2, 48h) (Compressive groups)
3. Human PDL cells that were obtained to mechanical vibration (0.3 g, at the frequency 30 & 60 Hz) 20 minutes every 24 h for 3 cycles of mechanical vibration. (Vibrated groups)
4. Human PDL cells that were subjected to continuous compressive forces (2 g/cm^2, 48 h) and mechanical vibration (0.3g, at the frequency 30 & 60 Hz) 20 minutes every 24 h for 3 cycles of mechanical vibration. (Compressive combined with vibraion groups)

All of sample groups were cultured in a humidified atmosphere of 5% CO_2 at 37°C. Immediately followed the completion of mechanical stimuli procedure, Real time-PCR for IL-1β and TNF-α were measured in cells of all groups.

RNA extraction and Real time polymerase chain reaction (Real time-PCR)

Total ribonucleic acid (RNA) from all human PDL cultured cells under different culture conditions were isolated immediately following the completion of mechanical stimulation procedure. The cells were lysed for RNA isolation by using an innuPREP DNA/RNA mini kit (analytic-jena, Germany). Reverse transcription turn total RNA to cDNA by using SuperScript III First-Strand Synthesis System (InvitrogenTM, USA). For each RNA sample, 1 μg was prepared by mixing substances from Tetro cDNA Synthesis kit according to manufacturer’s protocol. The template cDNA was created, prepared for real time-PCR. A semi-quantitative PCR was implemented on Rotor-Gene® Q (Qiagen, Germany) using My TaqTM DNA polymerase (Bioline Inc,USA). The amplified DNA was undergo electrophoresis on a 1.8% agarose gel and visualized by ethidium bromide fluorostraining by using a Molecular Imager® ChemiDocTM XRS+system with Image Lab (Bio-Rad Laboratories, Hercules, CA, USA) and analysed for presence, specificity, and length prediction.

Real time-PCR analyses were performed on Rotor-Gene® Q (Qiagen, Germany) by using SensiFAST™ SYBR No-ROX Kit (Bioline Inc, USA) (containing SYBR® Green I dye, dNTPs, stabilizers and enhancers) follow to the producer's protocol. The primers sequences of IL-1β, TNF-α and GAPDH are listed in Table 1. The amplication profile is one cycle. The polymerase activation start the PCR at 95 °C for 2 minutes, then denaturing at 95 °C 5 seconds, follow by annealing at 60 °C 10 seconds and extension at 72 °C 10 seconds for 40 cycles. During each cycle, The measurements were taken at annealing step termination at 60 °C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene in this study.

Table 1: Show sequence of the forward and reverse primers for Real-time polymerase chain reaction (Real-time-PCR)

Gene	**5'-forward primer-3'**	**5'-reverse primer-3'**
IL-1β	CACGCTCCGGGACTCACAGC	CTGGCCGCCTTTGGTCCCTC
TNF-α	TTGATGGCAGAGAGGAGGTTG	TTCTGCCTGCTGCACTTTGGA
GAPDH	ATGGTGGTGAAGACGCAGT	GCACCGTCAAGGCTGAGAAC

Enzyme-linked immunosorbent assay (ELISA)

IL-1β and TNF-α protein expression in culture medium were determined by ELISA methods according to manuals of ELISA kit protocol (DuoSet® ELISA Development kit;

R&D system, Minneapolis, MN, USA). In brief, 100 µl of supernatant from cultured cells were added to plates pre-coated with antibodies then incubated for 2 h at room temperature. After that, aspirated and washed three times by using Wash Buffer to remove excess conjugate and unbound sample. Add 100 µl of the Detection Antibody, diluted in Reagent Diluents, to each well and incubate 2 h at room temperature. Then, aspirated and washed three times by using Wash Buffer. Add working dilution of Streptavidin-HRP 100 µl to each sample's well and incubate 20 minutes at room temperature. Then, aspirated and washed three times by using Wash Buffer. Add Substrate Solution 100 µl to each sample's well and incubate 20 minutes at room temperature. The color development was stopped by add Stop Solution 50 µl to each sample's well and lightly tap the ELISA plate to protect thorough mixing. The absorbance was measured immediately by using a microplate spectrophotometer (Multiskan GO, Thermo scientific, USA) at wavelength 450 nm. The color concentration is directly proportional to the cytokine concentration in the samples.The results were calculated by comparing with standard curve. In brief, the standard curve was constructed by applying the mean absorbance to the plot Y axis and the X axis using the mean concentration. Moreover, best-fit curve was drawn through the point on the graph. If the sample was diluted, the mean concentration must be multiplied by the diluttion factor.

Statistical analysis

All data was presented as the means ± standard deviation of each group. The comparisons between groups were carried out by Kruskal-wallis test using a multiple comparison Mann-whitney U test post hoc test, with SPSS software, version 17.0, $p < 0.05$ was considered to indicate a significant difference.

CHAPTER 3

RESULTS

Cell culture and Identification of human periodontal ligament cells

A representative sample of spindle-shaped primary human PDL cells grown in culture is shown in Figure 9. To assess the formation of mineralized nodules, human PDL cells were cultured in osteogenic media and induced to differentiate into osteoblasts. The presence of alizarin red positive nodules form in human PDL cell culture after 14 and 21 days of induction is shown in Figure 10., which was indicated the accumulation of calcium and confirmed differentiation to osteoblasts.

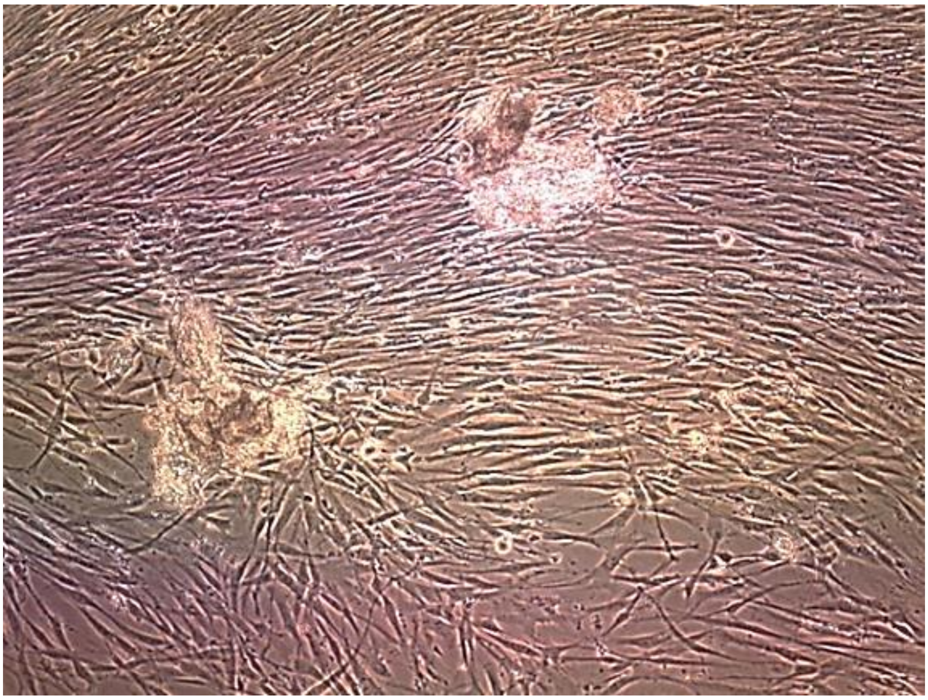


Figure 9: Spindle-shaped primary human PDL cells

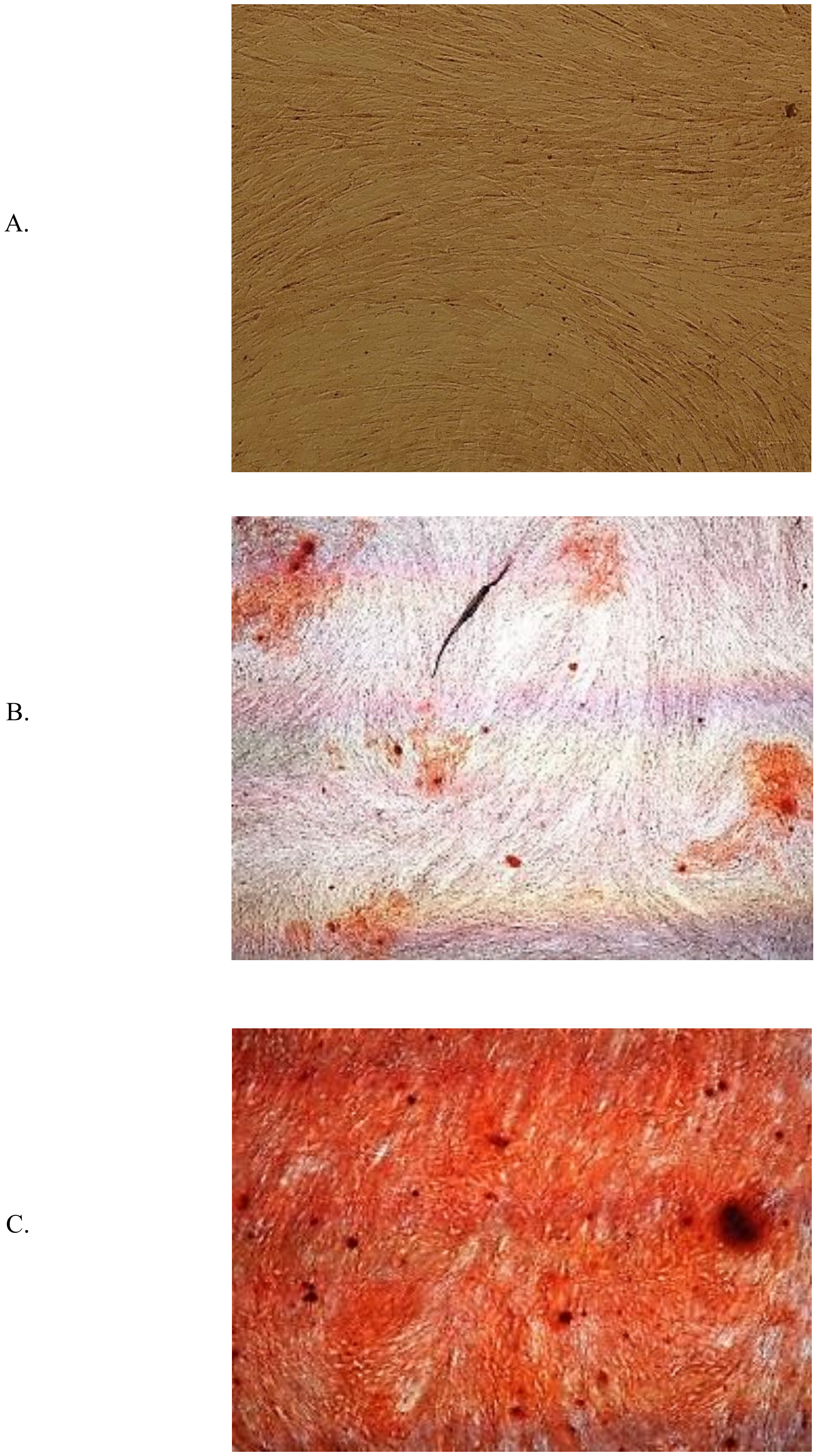


Figure 10: hPDL cells in DMEM for 21 days (A), hPDL cells in osteogenic medium for 14 days (B) and hPDL cells in osteogenic medium for 21 days (C)

IL-1β and TNF- α gene and protein expression levels in hPDL cells stimulated by mechanical vibration and compressive stress

The effect of mechanical vibration and compressive stress on cytokine expression in bone resorption was further verified by assessing the change in the expression of genes and proteins expression that associated with bone resorption. The mRNA expression of TNF-α was significantly increased in COM groups (4.3-fold increase compared with CT groups; $P < 0.05$) and CV groups both at 30 Hz and 60 Hz (5.82-fold and 2.67-fold increase compared with CT groups; $P < 0.05$, respectively) but decreased in all Vi groups (0.96-fold at 30 Hz and 0.75-fold at 60 Hz). However, there was no significant difference in the mRNA expression of TNF-α between Vi groups (30 Hz and 60 Hz) and CT groups. Interestingly, There were significant difference between Vi group versus COM groups and CV groups in all frequencies ($P < 0.05$) (Table 2 and Figure 11)

Table 2: The mRNA expression of TNF-α (Mean ± SD)

Groups	Mean of fold changes of TNF-α ± SD
CT	1.00 ± 0.76
COM	4.30 ± 2.06
Vi-30	0.96 ± 0.35
Vi-60	0.75 ± 0.31
CV-30	5.82 ± 1.59
CV-60	2.67 ± 1.35

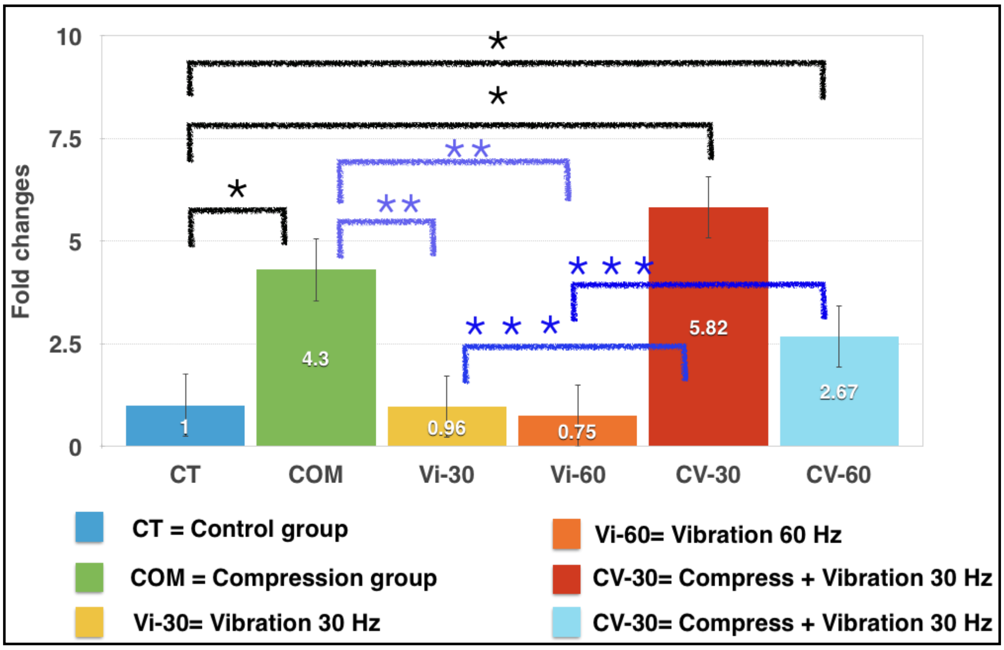


Figure 11: The mRNA expression of TNF-α in experimental and control groups.

Error bar: ± 1SD, Significance between CT groups and experimental groups, calculated with Kruskal-wallis test * ($P < 0.05$), Significance between COM groups and Vi groups in all frequencies, calculated with Kruskal-wallis test ** ($P < 0.05$), Significance between Vi groups and CV groups in each frequencies, calculated with Kruskal-wallis test *** ($P < 0.05$)

Similarly, with the mechanical vibration and compressive stress treatment, the mRNA expression of IL-1β showed significantly increased in COM groups (5.92-fold increase compared with CT groups; $P < 0.05$) and CV groups in all frequencies (8.83-fold and 2.99-fold increase compared with CT group; $P < 0.05$, respectively.) but decreased in all Vi groups (0.8-fold at 30 Hz and 0.71-fold at 60 Hz). However, there was no significant difference in the mRNA expression of IL1-β between Vi groups and CT groups. Moreove, the mRNA expression of IL-1β of Vi-groups significantly lower than COM groups and CV groups in all frequencies ($P < 0.05$). When compared the mRNA expression of TNF-α and IL-1β between 30 Hz and 60 Hz, there were no significant differnce both in Vi groups and CV groups ($P < 0.05$). However, the mRNA expression of TNF-α and IL-1β at 30 Hz are still higher than at 60 Hz both in Vi groups and CV groups. (Table 3 and Figure 12).

Table 3: The mRNA expression of IL-1β (Mean ± SD)

Groups	**Mean of fold changes of IL-1β ± SD**
CT	1.00 ± 0.94
COM	5.92 ± 3.02
Vi-30	0.80 ± 0.23
Vi-60	0.71 ± 0.18
CV-30	8.83 ± 3.97
CV-60	2.99 ± 1.70

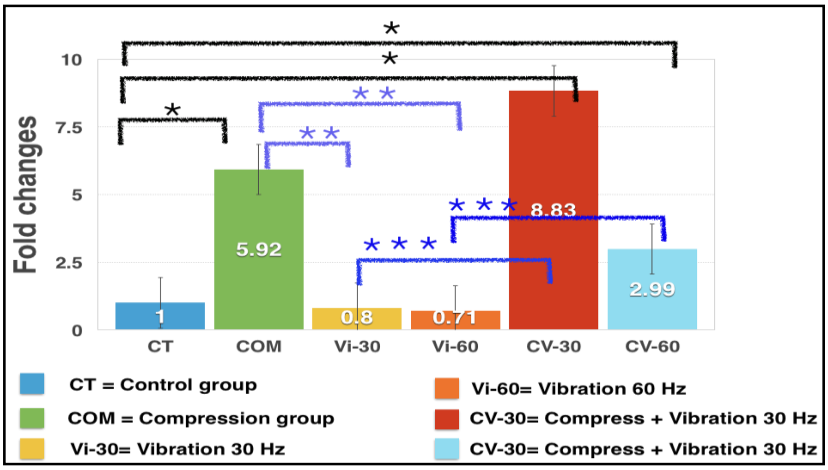


Figure 12: The mRNA expression of IL-1β in experimental and control groups. Error bar: ± 1SD, Significance between CT groups and experimental groups, calculated with Kruskal-wallis test *(P < 0.05), Significance between COM groups and Vi groups in all frequencies, calculated with Kruskal-wallis test ** (P < 0.05), Significance between Vi groups and CV groups in each frequencies, calculated with Kruskal-wallis test *** (P < 0.05)

In addition to the mRNA levels for TNF-α and IL-1β, their protein expression levels were measured by ELISA showed no significant difference in the protein expression of TNF-α and IL-1β in experimental and control groups ($P < 0.05$) (Table 4, 5 and Figure 13).

Table 4: The protein expression of TNF-α (Mean ± SD)

Groups	TNF-α/Total protein (pg/mg) ± SD
CT	1.63 ± 0.69
COM	1.68 ± 0.58
Vi-30	1.62 ± 0.49
Vi-60	1.71 ± 0.49
CV-30	1.66 ± 0.69
CV-60	1.74 ± 0.52

Table 5: The protein expression of IL-1β (Mean ± SD)

Groups	IL-1β/Total protein (pg/mg) ± SD
CT	81.67 ± 31.97
COM	100.79 ± 39.98
Vi-30	86.99 ± 31.87
Vi-60	90.37± 24.69
CV-30	90.26 ± 33.31
CV-60	121.88 ± 102.10

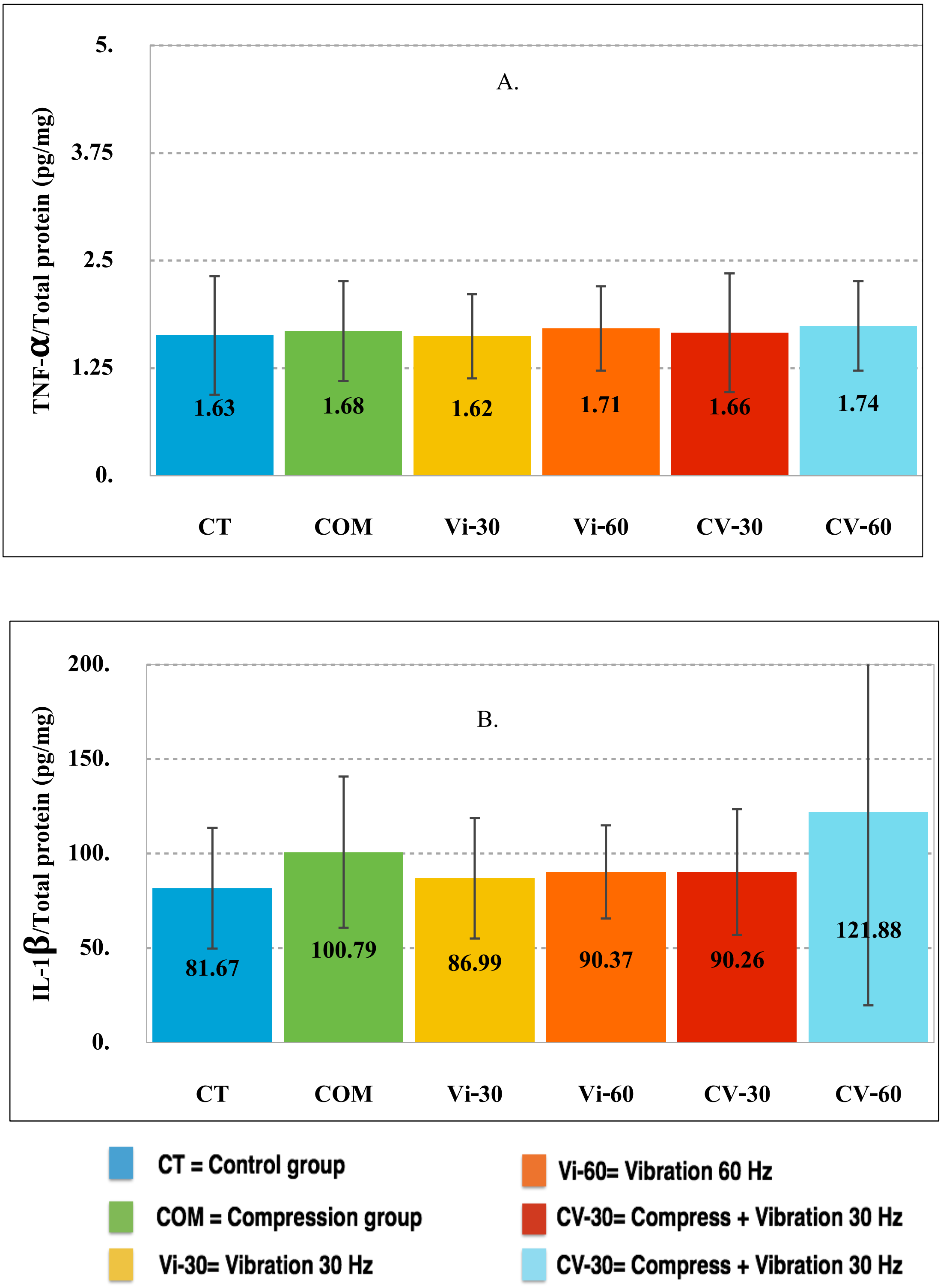


Figure 13: The protein expression of TNF-α (A.) and IL-1β (B.) in experimental and control groups. Error bar: ± 1SD, No statistical difference between control and experimental group ($p<0.05$)

CHAPTER 4

DISCUSSION

Currently, there are several methods used to accelerate orthodontic tooth movement. One of them is the use of mechanical vibration. However, the mechanisms of accelerated tooth movement by mechanical vibration are still unclear. Here, we aimed to investigate the underlying cellular and molecular mechanisms which the mechanical vibration plays an important role on the acceleration of orthodontic tooth movement in the compression side of PDL cells. From the results, the expression of IL-1β and TNF-α mRNA in the compression groups increased significantly higher than the control groups. This is consistent with the study of Kanzaki *et al.* [49] found that highest expression of RANKL mRNA when PDL cells were subjected to compressive force 2 g/cm^2 for 48 h. In addition, IL-1β, TNF-α and RANKL also have the same effects on osteoclastogenesis. Furthermore, There are studies found that IL-1β and TNF-α mRNA was significantly higher expression on the compression side than the tension side [11, 12]. It has been shown that IL-1β and TNF-α play the important role in bone resorption.

However, the mRNA expression of IL-1β and TNF-α decreased significantly in the mechanical vibration when compared with the compression group and control groups. Besides, there was no study of the cellular and molecular response to mechanical vibration in PDL cells. Therefore, these results may be explained by the study of Tanaka *et al.*[105] have been found that mechanical vibration favorably influences to osteoblast and osteoblast precursors. In addition, some studies [106-108] have shown that mechanical vibration changes the bone marrow stem cells into osteogenesis rather than adipogenesis, and also increases the osteoblastic activity. All of above-mentioned can be concluded that mechanical vibration stimulated bone formation. Furthermore, this is consistent with the study of Zhang *et a*l., [29] shown that mechanical vibration increased the expression of Runx2 and Osx Mrna which are the osteogenetic marker in human periodontal stem cells.

On the other hand, the expression of IL-1β and TNF-α mRNA significantly increased when vibration combined with compressive stress compares to the control and vibration groups. Nishimura *et al.,*[25] found that mechanical vibration with orthodontic force increased expression of RANKL and increased 1.7 times in osteoclast number. Moreover, Leethanakul *et al.,*[27] studied by using rotating and vibrating force with orthodontic force found the increasing of IL-1β levels in GCF and the tooth movement was accelerated. Although, the studies showed that mechanical vibration could accelerate tooth movement, there are some studies showed the opposite results of mechanical vibration with orthodontic force which was inhibit orthodontic tooth movement or no any effect on its[109] such as the clinical study that using a frequency of 111 Hz for 20 minutes per day about 10 weeks found that there was no differences in the rate of tooth movement. In addition to the study of woodhouse *et al.,*[110] studied by using Acceledent® for vibration of 30 Hz 20 minutes per day and found the same results in previous studies which no difference in the rate of tooth movement. Kalajzic *et al*[28] showed that cyclical force inhibited tooth movement due to 30-Hz cyclical forces inhibited osteoclastogenesis and decreased in osteoclast. In conclusion, the effect of mechanical vibration on the tooth movement varied in each studies due to the type of cells in the study. Some studies[29, 105] used periodontal ligament stem cells (PDLSCs), bone marrow stem cells (BMSCs) or osteoblasts. And in this study we used as PDL fibroblasts. In addition, each studies [27, 28, 29, 109, 97] also were different in the mechanical regimen (duration, magnitude and frequency), such as 20 minutes or 30 minutes per day. In addition, the total duration usages were varied from 3 days to 2 months. Moreover, the frequencies were varied from 10 Hz to 125 Hz including the different magnitudes of vibration may cause the different results.

Moreover, compression force from compressive stress model placed over the PDL cells cause compressed the cells in culture media and distributed the culture medium too high which lead to hypoxia. Therefore, this alteration stimulated the acute inflammatory process. According to the study[111] shown that hypoxia initiated acute inflammatory process and increased the expression of IL-1β, TNF-α, IL-6, IL-8 and VEGF in PDL fibroblasts. Taken together, physical strain generated the creation of these cytokines in PDL cells. All of above-mentioned lead to increase IL-1β and TNF-α mRNA expression in vibration combined with compressive stress groups [4].

In addition, there were no significantly different of IL-1β and TNF-α expression between 30 and 60 Hz frequency. However, when the 30 Hz frequency in range, there was an increased in IL-1β and TNF-α mRNA expression higher than at 60 Hz. Because the amount of force is too high or 60 H is not the proper frequency to stimulate IL-1β and TNF-α mRNA expression.

The protein expression, there was no significant difference in both IL-1β and TNF-α may be due to delay mRNA translation. Furthermore, the studies on the presence of increased IL-1β and TNF-α are based on in vivo study, which environmental condition difference from the in vitro study.

Limitation of the study

Even though the results from the less complicated nature of in vitro study cannot be directly replicate that of in vivo study due to the difference in environmental condition, this study had contributed some basic knowledge's on the use of vibration

Suggestion

Further study could focus on various cytokines that also involve in bone resorption process.

CHAPTER 5

CONCLUSIONS

This study demonstrated that compression force and mechanical vibration combined with compression force increase IL-1β and TNF-α expression in human periodontal ligament cells and highest the expression of IL-1β and TNF-α at 30 Hz of mechanical vibration combined with compression force. However, Mechanical vibration without compression force had no effect on the IL-1β and TNF-α expression in human periodontal ligament cells.

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APPENDICES

เอกสารชี้แจงและแบบยินยอมเข้าร่วมการศึกษา

ชื่อโครงการ	การประเมินทางห้องปฏิบัติการของการแสดงออกของยีน IL-1β และ TNF-α ในเซลล์สร้างเส้นใยของเอ็นยึดปริทันต์ภายหลังจากการได้รับแรงสั่นร่วมกับแรงกด
ชื่อผู้วิจัย	นางสาวบุณฑริกา อู่นาท นักศึกษาหลังปริญญาสาขาวิชาวิทยาศาสตร์สุขภาพช่องปาก (ทันตกรรมจัดฟัน) ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

เนื่องจากการรักษาทางทันตกรรมจัดฟันต้องใช้ระยะเวลาที่ยาวนานซึ่งอาจก่อให้เกิดผลเสียต่อผู้รับการรักษามากขึ้นตามระยะเวลาการรักษาที่ยาวนานมากขึ้นปัจจุบันจึงได้มีการคิดค้นวิธีใหม่ๆเพื่อนำมาใช้ในการ กระตุ้นการเคลื่อนฟันให้เร็วขึ้น เพื่อลดระยะเวลาในการรักษา และลดผลเสียที่อาจจะเกิดขึ้นจากการรักษาที่ยาวนานลง

การใช้แรงสั่นสะเทือนขนาดต่ำความถี่สูงเป็นหนึ่งในวิธีการที่นำมาใช้กระตุ้นการเคลื่อนฟัน โดยมีรายงานการศึกษาที่แสดงให้เห็นว่าแรงสั่นสะเทือนขนาดต่ำความถี่สูงสามารถเพิ่มอัตราการเคลื่อนฟันได้โดยไม่ก่อให้เกิดอันตราย นอกจากนี้ยังเป็นวิธีที่ใช้ง่ายเมื่อเทียบกับวิธีอื่นๆ แต่เนื่องจากปัจจุบันยังไม่มีรายงานการศึกษาใดที่สามารถอธิบายได้ว่า การกระตุ้นด้วยแรงสั่นสะเทือนขนาดต่ำความถี่สูงสามารถกระตุ้นการเคลื่อนฟันได้ผ่านทางการรับรู้ของเซลล์ใดและผ่านทางกลไกใดในระดับเซลล์

ดังนั้นการศึกษาในครั้งนี้ จึงมุ่งศึกษาผลของการกระตุ้นด้วยแรงสั่นร่วมกับแรงกดต่อการแสดงออกของยีนในเซลล์สร้างเส้นใยของเอ็นยึดปริทันต์ เพื่อจำลองลักษณะของเนื้อเยื่อปริทันต์ที่ได้รับการกระตุ้นด้วยแรงสั่นสะเทือนขนาดต่ำความถี่สูงร่วมกับแรงทางทันตกรรมจัดฟันเพื่อสามารถนำเอาความรู้ที่ได้มาอธิบายกลไกการตอบสนองในระดับเซลล์ และเป็นความรู้พื้นฐานในการสนับสนุนการใช้แรงสั่นสะเทือนขนาดต่ำความถี่สูงเพื่อกระตุ้นการเคลื่อนฟัน ลดระยะเวลาในการรักษาทางทันตกรรมจัดฟัน

โครงการวิจัยนี้จำเป็นต้องใช้เซลล์เนื้อเยื่อปริทันต์ที่ได้จากฟันกรามน้อยที่จำเป็นต้องถอนเพื่อการรักษาทางทันตกรรมจัดฟันตามปกติ จากนั้นนำเนื้อเยื่อปริทันต์มาเพาะเลี้ยง

และศึกษาในห้องปฏิบัติการ เพื่อให้ได้เซลล์สร้างเส้นใยของเอ็นยึดปริทันต์ โดยฟันกรามน้อยดังกล่าวเป็นฟันที่จำเป็นต้องได้รับการถอนตามมาตรฐานการรักษาปกติจากการรักษาทางทันตกรรมจัดฟัน เช่น การถอนฟันกรามน้อยเพื่อแก้ไขฟันซ้อนเก หรือเพื่อลดความยื่นของฟัน เป็นต้น การเข้าร่วมโครงการวิจัยนี้ไม่มีส่วนเกี่ยวข้องกับการถอนฟันออกมาเพิ่มเติมจากการรักษามาตรฐาน และเมื่อเสร็จสิ้นโครงการวิจัยนี้แล้ว ฟันกรามน้อยทั้งหมดจะถูกทำลายตามมาตรฐานของโรงพยาบาลทันตกรรม คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ส่วนเซลล์สร้างเส้นใยของเอ็นยึดปริทันต์ที่ได้จากการเพาะเลี้ยงเนื้อเยื่อปริทันต์จะถูกเก็บเพื่อใช้ในการศึกษาวิจัยในอนาคต โดยในกรณีที่นักวิจัยจะนำเซลล์สร้างเส้นใยของเอ็นยึดปริทันต์ที่ได้จากการเพาะเลี้ยงเนื้อเยื่อปริทันต์ของผู้เข้าร่วมโครงการไปใช้ศึกษาอื่นใด นอกเหนือจากที่ระบุไว้ในโครงการวิจัยนี้ จะต้องได้รับการพิจารณาจากคณะกรรมการจริยธรรมในการวิจัยในมนุษย์ คณะทันตแพทยศาสตร์ ก่อนดำเนินการโครงการวิจัย

ไม่ว่าท่านจะเข้าร่วมในโครงการวิจัยนี้หรือไม่ ท่านจะยังคงได้รับการรักษาที่ดีตามมาตรฐานการรักษาปกติเช่นเดียวกับผู้ป่วยคนอื่นๆ และถ้าท่านต้องการที่จะถอนตัวออกจากการศึกษานี้เมื่อใด ท่านก็สามารถกระทำได้อย่างอิสระ

หากท่านมีคำถามใด ๆ ก่อนที่จะตัดสินใจเข้าร่วมโครงการนี้ โปรดซักถามคณะผู้วิจัยได้อย่างเต็มที่

หากมีข้อสงสัยเพิ่มเติมที่เกี่ยวข้องกับโครงการวิจัย สามารถติดต่อได้ที่ ทพญ.บุณฑริกา อู่นาท เบอร์โทรศัพท์ 083-8755573 หรือ e-mail address: tuniezzzdent@gmail.com หรือ รศ.ดร.ทพญ. ชิดชนก ลีธนะกุล ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ เบอร์โทรศัพท์ 074-429875

หากผู้วิจัยมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ข้าพเจ้าทราบอย่างรวดเร็วโดยไม่ปิดบัง

ข้าพเจ้ามีสิทธิที่จะของดการเข้าร่วมโครงการวิจัยโดยมิต้องแจ้งให้ทราบล่วงหน้า โดยการงดการเข้าร่วมการวิจัยนี้ จะไม่มีผลกระทบต่อการได้รับบริการหรือการรักษาที่ผู้อยู่ภายใต้การปกครองของข้าพเจ้าจะได้รับแต่ประการใด

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับผู้อยู่ภายใต้การปกครองของข้าพเจ้า

เป็นความลับ จะไม่เปิดเผยข้อมูลหรือผลการวิจัยของผู้อยู่ภายใต้การปกครองของข้าพเจ้า เป็นรายบุคคลต่อสาธารณชน จะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย หรือการเปิดเผยข้อมูลต่อผู้มีหน้าที่ที่เกี่ยวข้องกับการสนับสนุนและกำกับดูแลการวิจัย

ข้าพเจ้าได้อ่าน/ได้รับการอธิบายข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจโดยนักวิจัยได้ให้สำเนาแบบยินยอมที่ลงนามแล้วกับข้าพเจ้าเพื่อเก็บไว้เป็นหลักฐานจำนวน 1 ชุด

ลงชื่อ…………..……….……..ผู้ยินยอม
ลงชื่อ…………………………บิดา/มารดา/ผู้ปกครอง
ลงชื่อ……………………......หัวหน้าโครงการ
ลงชื่อ…………………………พยาน
ลงชื่อ…………………………พยาน

หมายเหตุ: ผู้เข้าร่วมโครงการที่ยังไม่บรรลุนิติภาวะและสามารถเขียนหนังสือได้ให้เซ็นชื่อยินยอมเข้าร่วมโครงการด้วย

In vitro mineralization assay

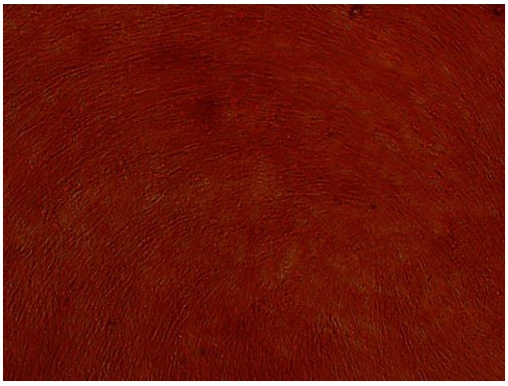
Sample 1

PDL in DMEM

PDL in osteogenic media

Sample 2

PDL in DMEM

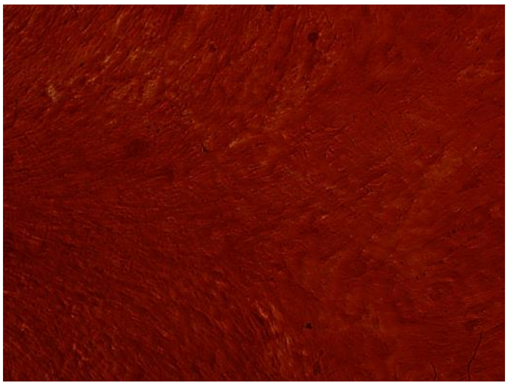


PDL in osteogenic media

In vitro mineralization assay

Sample 3

PDL in DMEM



PDL in osteogenic media

Sample 4

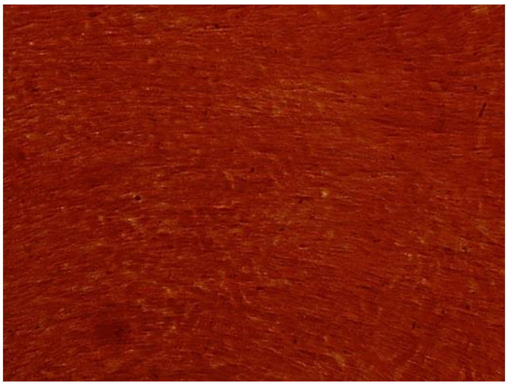
PDL in DMEM

PDL in osteogenic media

In vitro mineralization assay

Sample 5

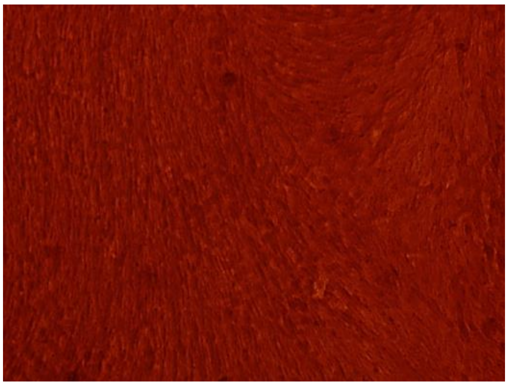
PDL in DMEM



PDL in osteogenic media

Sample 6

PDL in DMEM



PDL in osteogenic media

mRNA expression of IL-1β and TNF-α

Sample 1:

Groups	Fold changes compared with GADPH	
	IL-1β	TNF-α
CT	1	1
COM	5.22	4.41
Vi-30	0.83	1.01
Vi-60	0.82	0.41
CV-30	8.71	6.99
CV-60	2.19	2.10

Sample 2:

Groups	Fold changes compared with GADPH	
	IL-1β	TNF-α
CT	1	1
COM	5.50	5.70
Vi-30	0.83	1.07
Vi-60	0.78	0.97
CV-30	6.24	5.66
CV-60	2.67	4.12

mRNA expression of IL-1β and TNF-α

Sample 3:

Groups	Fold changes compared with GADPH	
	IL-1β	TNF-α
CT	1	1
COM	8.86	3.21
Vi-30	0.91	1.04
Vi-60	0.83	0.93
CV-30	13.82	4.53
CV-60	5.28	2.38

Sample 4:

Groups	Fold changes compared with GADPH	
	IL-1β	TNF-α
CT	1	1
COM	3.25	4.62
Vi-30	0.71	0.91
Vi-60	0.62	0.77
CV-30	8.01	6.70
CV-60	2.05	2.58

mRNA expression of IL-1β and TNF-α

Sample 5:

Groups	Fold changes compared with GADPH	
	IL-1β	TNF-α
CT	1	1
COM	7.98	3.84
Vi-30	0.86	1.12
Vi-60	0.76	0.86
CV-30	12.53	4.68
CV-60	5.43	2.40

Sample 6:

Groups	Fold changes compared with GADPH	
	IL-1β	TNF-α
CT	1	1
COM	3.23	3.56
Vi-30	0.35	0.81
Vi-60	0.25	0.68
CV-30	3.94	5.24
CV-60	2.77	2.19

VITAE

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Educational Attainment

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DDS.	Naresuan University	2009

List of Publication and Proceeding

Buntarika Unat, Chidchanok Leethanakul. Effect of Mechanical Vibration Combined with Compressive Stress the Expression of TNF-α in Human PDL Cells. The 7th Conference on Graduate Research, Southern College of Technology, February 24, 2017.