Change in Rate of Orthodontic Tooth Movement and Interleukin-1 beta Level in Gingival Crevicular Fluid in Response to Mechanical Vibratory Stimulation from Electrical Toothbrush

Sumit Suamphan

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Author Mr. Sumit Suamphan

Major Program Oral Health Sciences

Major Advisor :

……..

(Assoc. Prof. Dr. Chidchanok Leethanakul)

Co-advisor :

…… …….

(Assoc. Prof. Dr. Chairat Charoemratrote)

……

(Asst. Prof. Dr. Suwanna Jitpukdeebodintra)

Examining Committee :

……..Chairperson

(Prof. Smorntree Viteporn)

……Committee

(Assoc. Prof. Dr. Chidchanok Leethanakul)

……..…… Committee

(Assoc. Prof. Dr. Chairat Charoemratrote)

……..…… Committee

(Asst. Prof. Dr. Suwanna Jitpukdeebodintra)

……..…… Committee

(Asst. Prof. Dr. Wilairat Worapamorn)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Sciences Degree in Oral Health Sciences

……..……..

(Assoc. Prof. Dr. Krerkchai Thongnoo)

Dean of Graduate School

ชื่อวิทยานิพนธ์ การเปลี่ยนแปลงอัตราเร็วในการเคลื่อนฟันและระดับอินเตอร์ลูคิน 1 ชนิดเบต้าในน้ำเหลืองเหงือกระหว่างการเคลื่อนฟันทางทันตกรรมจัดฟันร่วมกับการใช้แรงสั่นสะเทือนจากแปรงสีฟันไฟฟ้า

ผู้เขียน นายสุมิตร สูอำพัน

สาขาวิชา วิทยาศาสตร์สุขภาพช่องปาก

ปีการศึกษา 2552

บทคัดย่อ

การเพิ่มอัตราเร็วในการเคลื่อนฟันสามารถช่วยลดระยะเวลาการรักษาและภาวะอันไม่พึงประสงค์ที่อาจเกิดขึ้นในการรักษาทางทันตกรรมจัดฟัน ทั้งนี้การเพิ่มอัตราเร็วในการเคลื่อนฟันควรอาศัยวิธีการที่เรียบง่าย ไม่ยุ่งยากซับซ้อน การศึกษาในครั้งนี้จึงทำการศึกษาความเป็นไปได้ในการใช้แรงสั่นสะเทือนที่ได้จากแปรงสีฟันไฟฟ้ามาช่วยเพิ่มอัตราการเคลื่อนฟัน **วัตถุประสงค์** เพื่อศึกษาผลของการใช้แรงสั่นสะเทือนจากแปรงสีฟันไฟฟ้าร่วมกับแรงทางทันตกรรมจัดฟันต่อการเปลี่ยนแปลงอัตราการเคลื่อนที่ของฟันและระดับตัวบ่งชี้ทางชีวภาพภายในน้ำเหลืองเหงือก **วิธีการวิจัย** กลุ่มตัวอย่าง 10 คน (ชาย 2 คน, หญิง 8 คน อายุเฉลี่ย 20.5 ปี) ซึ่งจำเป็นต้องถอนฟันกรามน้อยซี่ที่ 1 บนทั้งซ้ายและขวาเพื่อการเคลื่อนฟันเขี้ยวไปด้านไกลกลาง ฟันเขี้ยวข้างหนึ่งจะได้รับการกระตุ้นด้วยแรงสั่นสะเทือนร่วมกับแรงทางทันตกรรมจัดฟันขนาด 60 กรัม เป็นเวลา 1 เดือน เปรียบเทียบกับฟันเขี้ยวอีกด้านซึ่งได้รับแรงทางทันตกรรมจัดฟันเพียงอย่างเดียว ในการนัดหมายแต่ละครั้งแบบจำลองฟันและน้ำเหลืองเหงือกที่ได้จากผู้ป่วยจะถูกนำมาวัดเพื่อหาอัตราการเคลื่อนฟันและระดับอินเตอร์ลูคิน 1 ชนิดเบต้าตามลำดับ **ผลการศึกษา** อัตราเร็วในการเคลื่อนฟันเขี้ยวในกลุ่มทดลองสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติที่ระดับความเชื่อมั่น 95% ระดับอินเตอร์ลูคิน 1 ชนิดเบต้าในกลุ่มทดลองสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติที่ระดับความเชื่อมั่น 95% ทั้งนี้ไม่พบภาวะแทรกซ้อนทางคลินิก **สรุปผลการศึกษา** แรงสั่นสะเทือนสามารถเพิ่มอัตราการเคลื่อนฟันทางทันตกรรมจัดฟันร่วมกับมีการเพิ่มปริมาณอินเตอร์ลูคิน 1 ชนิดเบต้าในน้ำเหลืองเหงือก

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ABSTRACT

Accelerating the rate of orthodontic tooth movement would be beneficial to the shortening of treatment time and to diminish adverse effects in orthodontic treatment. The acceleration of tooth movement should be achieved by new simple technique without risks and harmful procedure. **Objectives** In this study, the effects of mechanical stimulation by vibration were evaluated on tooth movement and cytokine secretion. **Materials & Methods** Ten subjects (2 males and 8 females, mean age 20.5 years) who received the upper first premolar extraction from both sides for canine distalization were participated. With the split mouth design, canine on the experimental site was applied with orthodontic force (60 g) in combination with the vibratory stimulation from the electrical toothbrush for 1 month compared to orthodontic force alone. The amount of canine movement was measured from the study model. The gingival crevicular fluid was collected from both mesial and distal region of each canine at each appointment. IL-1β level was analyzed by ELISA. **Results** Tooth movement in the experimental group was significantly ($p<.05$) greater than in the control group. Enhanced IL-1β secretion was observed in the distal site of the experimental group after vibratory stimulation for 1 month which was significantly ($p<.05$) increased over the control group. Clinically, there were no pathological findings in either group. **Conclusion** The application of vibration in this study can accelerate orthodontic tooth movement via enhanced IL-1β secretion in the gingival crevicular fluid.

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LIST OF ABBREVIATION AND SYMBOLS

GCF	= Gingival crevicular fluid
IL-1β	= Interleukin 1-beta
IL-8	= Interleukin 8
NSAIDs	= Nonsteroidal anti-inflammatory agents
mRNA	= Messenger ribonucleic acid
PDL	= Periodontal ligament
RANK	= Receptor activator of nuclear factor kappa B
RANKL	= Receptor activator of nuclear factor kappa B ligand
OPG	= Osteoprotegerin
ELISA	= Enzyme-linked immunosorbent assay
PCR	= Polymerase chain reaction
PBS	= Phosphate buffered saline
kDa	= Kilo-Dalton
Hz	= Herz
NiTi	= Nikle Titanium
°C	= Celsius
µl	= Microliter
mm	= Millimeter
nm	= Nanometer
pg/ml	= Picogram per millimeter
T0	= start canine retraction
T1	= 1 month after canine retraction
T2+V1	= 2 months after canine retraction with or without 1-month vibration
T2+V1	= 2 months after canine retraction with 1-month vibration
T2-V1	= 2 months after canine retraction without 1-month vibration
T3+V2	= 3 months after canine retraction with or without 2-month vibration
T3+V2	= 3 months after canine retraction with 2-month vibration
T3-V2	= 3 months after canine retraction without 2-month vibration

CHAPTER 1

INTRODUCTION

Background and Rationale

Orthodontic tooth movement is the result of periodontal tissue response to the mechanical stimuli generated from orthodontic appliances that alter the homeostatic condition causing the transient change, resulting in tooth movement into the more equilibrium environment through periodontal tissue adaptation including bone remodeling. This process involves in bone cell activities regulated via many signaling pathways and mechanisms.[1] The acceleration of orthodontic tooth movement is brought about by raising the remodeling activity of bone. The velocity of orthodontic tooth movement is related to cytokine release which can be detected in gingival crevicular fluid (GCF).[2] Among these cytokines, interleukin 1-beta (IL-1β) is one of the potent inflammatory mediator that showed positive correlation to faster tooth movement.[3]

Triggering the bone remodeling process for the aim of increase in rate of tooth movement can be done to provide each patient with the benefits of less treatment time including administration of inflammatory agents,[4,5] electrical stimulation,[6,7] laser application,[8,9] and also surgical enhancement of tooth movement.[10, 11] Some of these methods can increase movement speed according to the literatures. However, they are not yet the common procedures base on the concern in unsatisfied effects and further studies in human are necessary.

The rate of tooth movement apparently depends on the magnitude and duration of force.[12] However, the up-regulation of bone remodeling by keeping the force increase is absolutely useless. According to the tissue tolerance, the hyalinization occurs followed by prolonged treatment time when the force is beyond the optimum limit.[13] Ideal orthodontic treatment requires an optimum force that leads to the maximum rate of tooth movement with minimal irreversible damage to the root and periodontium.[14] However, a large inter-individual variation was found in experiment attempted to define the optimum force. Even with

standardize, constant and equal force, the rate of orthodontic tooth movement may vary substantially among and even within individuals.[15-17]

Accordingly, the light magnitude force was introduced for satisfied result by avoidance of necrotic periodontal tissue. Unfortunately, the maximum biologic response may not be achieved by low magnitude in some individuals.[2, 3]

Any assault on up-regulated bone remodeling process with non-aggressive mechanical stimulation was showed in studies that meant to reduce the incidence of osteoporosis by using the principle of short duration, extremely small magnitude, high-frequency mechanical stimuli.[18-22] The results illustrated the significantly up-regulated bone remodeling activities due to increase in capillary filtration and number of blood vessels. In addition, vibratory stimulation applied in animal model was shown to increase rate of orthodontic tooth movement without additional damage to tissues nearby.[23] The osteocytes might be the one that believed to act as the mechano-sensor of the bone. Within the lacunar space in bone, the cytoplasmic processes of osteocytes and contained bone fluid are evidently responsible for mechanical signal transduction to surrounding osteocytes and bone lining osteoblasts through the lacunocanalicular network.[24-27]

In this study, the vibratory stimulation is hypothesized to promote the velocity of tooth movement via the combination of light orthodontic force and vibratory stimulation in clinical practice. By using an already available instrument for tooth cleansing, the electrical toothbrush, is the oral hygienic device that uses electric power to generate oscillatory pattern, which in term creates the vibratory motion to the brush head that result in up-regulation of the mechanical signals for alveolar bone remodeling.

Review of Literature

Optimal orthodontic treatment requires a mechanical input that leads to a maximum rate of tooth movement with minimal irreversible damage to the root and periodontium. It is assumed that an optimal force system is important for an adequate biological response in the periodontal ligament. Throughout time, many studies are conducted to seek the optimum force for tooth movement. However, a large inter-individual variation is recognized in both human research and animal experiments. In literature, different opinions can be found about

the force level that results in optimal mechanical conditions within the periodontal ligament for orthodontic tooth movement.

Optimum force magnitude for orthodontic tooth movement

Recently, a thorough systematic study of the literature pertaining to the efficiency of tooth movement in human subjects indicated that no optimal force magnitude, or more accurately no optimal pressure magnitude, could be defined.[28] The main problems encountered are related to the inability to estimate stresses or stress distribution in the periodontal ligament, the lack of control of bodily or tipping movement, the variation in follow-up periods, and large individual variations.[29]

Regardless, this issue is generally based on the purposed models for the relationship between force magnitude and rate of tooth movement.

The best model that is supported by experimental and clinical data illustrate the force threshold is indicated for tooth movement initiation. With forces above the threshold, a linear dose-response relationship is assumed. The higher forces are generally more efficient in moving teeth. Then a plateau is reached, the higher forces do not necessarily lead to faster tooth movement.[29] A minimal force, leading to a minute change in pressure, might be able to switch on tooth movement. This implies that higher forces often used in orthodontic practice do not necessarily produce more efficient tooth movement. On the contrary, they might overload the periodontal tissues and cause negative effects that will delay tooth movement.

Mechanotransduction in bone biology and homeostasis

Mechanotransduction is the process by which mechanical energy is converted into electrical and/or biochemical signals. In principle, the loading information must then be communicated to effective cells that can make new bone or destroy old bone. Mechanical adaptation is a cellular process that senses the applied mechanical loading.[30] Osteoblasts are the cells that produce new bone by synthesizing bone matrix and making calcification. In the other hand, osteoclasts are the cells that can degrade bone matrix by subsequent demineralization and

collagen degradation. However, the great majority of the cells of bone tissue are osteocytes, lying within the bone matrix, bone lining cells and also lying on the surface. Osteocytes are derived from osteoblasts that have stopped producing bone matrix and later buried in bone matrix. Osteocytes remain in contact with the bone surface cells and with neighboring osteocytes via long slender cell processes that connect by means of gap junctions called canaliculi. Like a neuron, individual cytoplasmic process connects to surrounding osteocytes and superficial osteocytes also are connected with osteoblasts lined on the bone surface.[31]

At the tissue and cellular levels, mechanism of signal transduction in bone is still unclear. Somehow, in response to the external mechanical loading, bone cells may sense and transduce mechanical signals through the lacuno-canalicular network. According to the cell morphology and environment, the osteocytes might be the cell that believed to act as the mechanosensing of the bone.[25] Within the lacunar space, osteocytes and their processes are contained and filled with bone fluid. According to the canalicular fluid flow hypothesis, when bone is loaded, interstitial fluid is squeezed through the thin layer of non-mineralized matrix surrounding cell bodies and cell processes toward the Haversian or Volkmann channels, thereby producing fluid shear stress at the osteocyte cell membrane.

In steady state, fluid flow keeps the osteocytes viable and also ensures osteocyte activation and signaling, thereby suppressing osteoblastic activity as well as osteoclastic function. During overuse, the osteocytes are over-activated by enhanced fluid flow, leading to release of osteoblast-recruiting signals. Subsequent osteoblastic bone formation reduces the overuse until normal mechanical use is re-established, thereby re-establishing the steady state of basal fluid flow. During disuse, the osteocytes are inactivated by lack of fluid flow. Inactivation either leads to release of osteoclast-recruiting signals or to lack of osteoclast suppressing signals, or both. [30]

Vibratory stimulation and bone remodeling

Regarding to the response of osteocytes caused by stress-induced fluid flow stimulation, osteocytes are obviously sensitive to stress applied. There are many kinds of mechanical stress that might be sensed by osteocytes. In case of vibratory stimulation, vibration refers to mechanical oscillations about an equilibrium point with periodical change. The effects

of vibration on bone tissue/cells have widely been studied in the reason for treatment of osteoporosis. This concept arises from the extremely low-level, high-frequency strains on bone mass that caused by muscle contractibility during postural control which have recently been shown to be anabolic to bone tissue.[32]Animal studies indicate that low-magnitude high-frequency strains, induced through vibration, can stimulate bone formation in weight-bearing regions of the skeleton.[33, 34]

It would seem that higher frequencies are also stimulatory to bone cells. An investigation of bone cell responses to vibration stress at a wide frequency range (5–100 Hz) showed positively nitric oxide release correlated. COX-2 mRNA expression increased in a frequency-dependent manner, which relates to increased nitric oxide release at high frequencies. On osteoblastic function, osteocalcin mRNA was up-regulated 2.6-fold after 7 days of vibratory stimulation. Therefore, osteoblasts might be sensitized to mechanical loading by low amplitude, broad frequency mechanical vibration.[35]

In human studies, reported that having adult stand on a platform with high-frequency vibration for 20 minutes each day for 5 days a week over 1 year with load applied to bone from this vibration is about 5 microstrain could increase femoral trabecular bone density by 32%.[36] Bone trabeculae were also shown to have closer spacing, which was consistent with stronger bone. Histomorphometric studies of bone turnover suggested that this effect may due to the increase in bone formation and mineralization. The potential for brief periods of low-magnitude, high-frequency mechanical signals to enhance the musculoskeletal system was evaluated in young women with low bone marrow density. Twelve months of this non-invasive signal, induced as whole body vibration for at least 2 minutes each day, increased bone and muscle mass in the axial skeleton and lower extremities compared with controls.[37]

Periodontal ligament cells and mechanical stimulation

In homeostasis of alveolar bone, periodontal ligament (PDL) cells have intimate role in regulation of bone remodeling activities. PDL cells initiate bone remodeling in response to the load applied during orthodontic tooth movement.[38] On the pressure side, osteoclasts resorb bone. Osteoclast formation and differentiation are regulated by a balance between the receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG). PDL cells

express both RANKL and OPG. PDL fibroblasts are functionally different but may have dual effects. They are capable of inducing the formation of osteoclast-like cells and also inhibiting them according to the conditions examined.[39-42] It was shown that weak intermittent force could effectively induce the expression of RANKL in human PDL cells under optimal compression forces *in vitro*.[40, 42]

Vibratory stimulation and orthodontic tooth movement

The complex of tooth and PDL is considered to be viscoelastic. It was reported that an intermittent vibrating force is mechanically more effective than a static force in changing the PDL's viscoelasticity, and this effect persists over a certain period of time.[43] The ultrasonic vibration was used to accelerates tooth movement *in vivo*.[44] However, ultrasonic vibration of teeth might be associated with certain hazards, such as thermal damage to the dental pulp.

Interestingly, the effects of 60 Hz resonance vibration for 8 minutes on the speed of tooth movement and root resorption during experimental tooth movement in rats clearly demonstrated the stimulatory effects in accelerating the speed of tooth movement with no collateral damage to periodontal tissues.[23] In addition, the study demonstrated the activation of the RANK/RANKL signaling pathway in response to the loading of resonance vibration.

Furthermore, vibration was hypothesized to accelerate orthodontic tooth movement by alleviation of blood flow obstruction at the pressure side which may result in delayed tooth movement because of hyalinization.[23]

Gingival Crevicular Fluid (GCF)

From Dictionary of Dentistry Jablonski (1982) illustrated the meaning of GCF as a fluid occurring in minute amounts in the gingival crevice, believed by some authorities to be an inflammatory exudate and by others to cleanse material from the crevice, containing sticky plasma proteins which improve adhesions of the epithelial attachment, have antimicrobial properties, and exert antibody activity. GCF was discovered accidentally and had been described by Niels Brill and Bo Krasse in 1956.[45]

They observed the fluid oozing out of the periodontal pocket and washed out the idodine solution while the healthy gingival of a dog was disinfected for microflora collection. It is now widely appreciated that GCF is formed as a blood ultra-filtrate but accumulates elements of the metabolism from both bacterial and host cells from the gingival crevice environment. The interest of GCF is focused primarily on the identification of components that could be used to identify persons at risk in terms of periodontal disease activity. The significant function of GCF is an important determinant in the ecology of the periodontal sulcus. The first important characteristic associated with GCF flow is its flushing action. GCF flow creates a flushing action for the rapid removal of substances from the gingival sulcus. The other relevant characteristic associated with GCF flow is the isolation effects which restrict the penetration of substances from the periodontal pocket. In addition, the important role that GCF may have in transporting antibacterial substances, either of host origin or those obtained into the circulation such as antibiotics, to the crevicular space.[46]

The diagnostic potential of GCF flow has long been recognized. GCF flow rate is the process of fluid moving into and out of the gingival crevice in a small stream, usually only a few microliters per hour. The amount of crevicular fluid is correlated to the level of gingivitis. An increase in vascular permeability chronically inflamed gingival showed an increase in GCF production and periodontal therapy can result in a decrease in fluid flow.[47] A good correlation between GCF flow rate and the level of gingival inflammation and even higher correlation between flow rate and pocket depth had been demonstrated. In contrast, only a weak correlation between GCF flow and pocket depths had been described.[48]

GCF analysis

The composition of GCF has been a controversial issue. This may be a result of variations in the amount and nature of the fluid produced under different clinical conditions and the use of a wide variety of sampling methods. Problems with GCF collection and data interpretation mostly are caused by contamination. The major sources of contamination of GCF samples would be blood, saliva, or plaque. The presence of any contamination on filter paper strips used for collecting GCF would influence volume determinations, which would be expressed as volume of GCF. Careful isolation should be performed in an effort to minimize the

potential for contamination. Alternative approaches to sampling techniques have been developed in an effort to increase the volume of GCF sample available for subsequent laboratory analysis. The problem with prolonged collection times is that the nature of the GCF sample collected is likely to change with the protein concentration of the initial GCF collected comparable to interstitial fluid, whereas prolonged sampling at the site resulted in protein concentrations approaching those of serum.[49] In essence of volume determination, evaporation is considered to be a significant problem in accurate volume determination of GCF samples. This is particularly the case as the total volumes collected are usually less than 1 ml. When the small amount of GCF is collected, the percentage of error is considered to be of major significance.[48] There are conventional methods for measurement of GCF volume. Each approach collects the liquid sample and measure the GCF volume in related to time. Ideally, the method should provide short collecting time with adequate amount of GCF and less contamination from both environment and new GCF release. The key to this collection is to select a sampling time that is small enough so the resting volume of the pocket is not allowed to re-establish. The analysis of specific constituents in the GCF may provide quantitative biochemical indicators for using to evaluate the local cellular metabolism, reflecting the periodontal health status and bone remodeling process during orthodontic treatment. In recent years, the expression of regulatory proteins in the GCF has been recognized as a promising diagnostic tool for monitoring orthodontic treatment outcome. Currently, the information from individual studies on the relationship between regulatory proteins in GCF and orthodontic tooth movement is focused. The interest of most reports is pointed on the presence of new mediators and on the up-regulation/down-regulation of the levels of these regulatory proteins in different treatment designs.[46]

Cytokines in gingival crevicular fluid and orthodontic tooth movement

Cytokines are extracellular signaling proteins that act on nearby target cells in low concentrations in an autocrine or paracrine fashion in cell-to-cell communications. Among the more than 100 regulatory proteins detected in GCF, cytokines are proteins of interest. Cytokines refer to non-hormone signaling factors, including lymphocyte-derived factors, monocyte-derived factors, colony-stimulating factors, growth factors, and chemotactic

chemokines. Cytokines are produced by periodontal tissue cells, such as fibroblasts and osteoblasts, and are involved in normal physiological turnover and remodeling of bone.[50]

Pro-inflammatory cytokines have been intimately involved in bone resorbing activities. The elevation of the pro-inflammatory cytokines initiate signaling cascades that stimulate local vascularity, infiltration and activation of monocytes/macrophages, and the production of potent secondary pro-inflammatory mediators, chemoattractants, proteases, and reactive ions that collaborate to cause marked and irreversible bone breakdown. The role of cytokines in osteoclast formation and activation are central to the focal bone destruction that occurs in osteolytic conditions.[51]

Many characteristic features of cytokines make them particularly interesting to orthodontics. As cytokines in GCF, these mediators reflect the local microenvironment of periodontal tissues where the effect of orthodontic forces is exerted.[52] Numerous studies had shown that cytokines play pivotal roles in cell–cell signaling and mediate remodeling in bone following mechanical stimulation.[53-55] Clinical studies on the up-regulation/down-regulation of cytokines in GCF collected from patients have been carried out to provide a non-invasive way to show the involvement of cytokines in orthodontic tooth movement.[56-58] The relationship between cytokine production and force re-activations provide a better illustration of the high potential of GCF as a diagnostic tool for using to monitor clinical outcome in orthodontics. Cytokines that were found to affect bone metabolism, and thereby orthodontic tooth movement , such as interleukin 1, interleukin 6, interleukin 8, tumor necrosis factor alpha, and gamma interferon. Among the cytokine expressed in GCF, interleukin-1beta is one of the most studied in orthodontic literatures.

Interleukin-1beta (IL-1β)

Interleukins are a group of signaling molecules that were first seen to be expressed by white blood cells (leukocytes), The term interleukine, (*inter-*) as a means of communication, (*-leukin*) deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes.

IL-1 is the prototypic pro-inflammatory cytokine. There are two forms of IL-1, (IL-1α or IL-1β) functions as a costimulator of T cell functions, primarily together with an

antigen or a mitogen. IL-1α and IL-1β are synthesized as precursors without leader sequences. The molecular weight of each precursor is 31 kDa. Processing of IL-1α or IL-1β to mature forms of 17 kDa requires specific cellular proteases. There are two primary cell surface binding proteins (IL-1 receptors) for IL-1. IL-1 type 1 receptor (IL-1RI) transduces a signal, whereas the type 2 receptor (IL-1RII) binds IL-1 but does not transduce a signal. In fact, IL-1 RII acts as a sink for IL-1 and has been termed a decoy receptor. When IL-1 binds to IL-1RI, a complex is formed which then binds to the IL-1R accessory protein. (IL-1RAcP), resulting in high affinity binding.[59]

IL-1 is a key mediator in a variety of activities in immune and acute-phase inflammatory responses.[13] Both 2 forms of IL-1 (α and β) directly stimulate osteoclast function through IL-1 type 1 receptor, expressed by osteoclasts. Secretion of IL-1 is triggered by various stimuli, including neurotransmitters, bacterial products, other cytokines, and mechanical forces. These actions include attracting leukocytes and stimulating fibroblasts, endothelial cells, osteoclasts, and osteoblasts to promote bone resorption and inhibit bone formation. Osteoblasts are target cells for IL-1, which in turn conveys messages to osteoclasts for resorbing bone.[13] Between IL-1β and α, IL-1β is seem to be more potential. The expression of IL-1β mRNAs might be up-regulated in both PDL cells and osteoblasts on the compression side.[32] Many studies in human showed 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.[3, 56, 57] The deceleration of IL-1β level could be assumed that the restored IL-1β levels might be due to a lack of force consistency.[60] With respect to the velocity of tooth movement, there is a study that found a positive correlation with the IL-1β: IL-1 receptor antagonist ratio.

PDL cells and IL-1β

PDL cells produce IL-1β under mechanical stress.[61] The expression of IL-1β in the human periodontium can be assessed non-invasively by GCF analysis, which reflects the cellular response of underlying PDL space.[60] IL-1β-treated PDL cells showed an increase in bone resorbing activity compared with unstimulated PDL cells. IL-1β mediates osteoclastogenesis by enhancing stromal cell expression of RANKL and directly stimulating the

differentiation of osteoclast precursors.[62] Therefore, IL-1β probably modulates the bone remodeling caused by orthodontic compression forces.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay, also called ELISA, is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality control check in various industries. In simple terms, in ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal. Thus in the case of fluorescence ELISA, when light of the appropriate wavelength is shone upon the sample, any antigen/antibody complexes will fluoresce so that the amount of antigen in the sample can be inferred through the magnitude of the fluorescence. Performing an ELISA involves at least one antibody with specificity for a particular antigen. After the antigen is immobilized the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through conjugation. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. Traditional ELISA typically involves chromogenic reporters and substrates which produce some kind of observable color change to indicate the presence of antigen.

The generation of mechanical vibration from electrical toothbrush

An electrical toothbrush (Colgate® Motion-Multi Action) consisted of two round-tufts of brush which are rotated at approximately 7,500 rounds per minute (125 Hz) in opposite direction by a direct circuit (DC) motor which required two AAA alkaline batteries.

In a typical DC motor, there are permanent magnets on the outside and a spinning armature on the inside. The permanent magnets are stationary. The armature rotates.

The armature contains an electromagnet. As shown in Figure 5, when the electricity is run into this electromagnet, it creates a magnetic field in the armature that attracts and repels the magnets in the stationary unit. So the armature spins through 180 degrees. In order to keep it spinning, the poles of the electromagnet have to be changed. The brushes handle this change in polarity. They make contact with two spinning electrodes attached to the armature and flip the magnetic polarity of the electromagnet as it spins. The spinning of armature magnet generates the vibration as a by-product.

Objectives

1. To study the effect of vibratory stimulation on the rate of tooth movement.
2. To study the effect of vibratory stimulation on the IL-1β level in GCF.

Hypothesis

The vibratory stimulation can accelerate orthodontic tooth movement by increasing the remodeling activity of bone.

Significance of the study

This study introduces a new intervention for shorten the orthodontic treatment time with the simply method by using the vibratory stimulation from electrical toothbrush as the supplementary force which reduces cost and time spending in orthodontic treatment.

CHAPTER 2

Research Methodology

Subjects and Treatment

Ten patients (8 females and 2 males, age between 19-25 years) from the Orthodontic clinic, Faculty of Dentistry, Prince of Songkla University were involved in this study. The representative samples were selected from the population by simple random sampling following the criteria as below:

Inclusion Criteria

1. Good general health
2. Demonstrated good oral hygiene and had been planned to be extracted the maxillary first premolars and distalized the maxillary canines
3. Probing depth values not exceeding 3 mm. in the whole dentition
4. No NSAIDs taking within 6 months before the experiment

Exclusion Criteria

1. Loss of any permanent tooth during experiment (except the third molar)
2. Failure of bonding on the maxillary canine and/or movement unit
3. Present of periodontitis
4. Taking NSAIDs during the study

All patients were informed about the experimental procedure and the informed consent and assent were obtained according to the Institutional Ethical committee Board. The patients were instructed to avoid NSAIDs during the study. Acetaminophen was prescribed as necessary to alleviate discomfort from the orthodontic treatment. The patients received repeated oral hygiene instructions for the use of toothbrush and dental floss during the study.

Orthodontic mechanics

For each patient, Roth's prescription preadjusted edgewise brackets (3M Gemini metal brackets, 3M Unitek Corporation, Monrovia, CA, USA) with 0.022 x 0.025-inch slots were attached on maxillary canines and posterior teeth after the first premolars were extracted. For anchorage preparation, the upper second molar through second premolar were tied together with 0.010-inch stainless steel wire and bind bilaterally with 0.021 x 0.025-inch stainless steel wire segment to engage passively the tubes and slots of the edgewise appliances. The wires were left *in situ* for 1 month to become passive before starting to retract canines.

As shown in Figure 1, the mechanic for canine distalization consisted of a customized retraction hook fabricated from 0.021 x 0.025-inch stainless steel arch wire on the mesial of each canine and second premolar bracket. The height of the hook was just matched the estimated center of resistance (CRE) position for the specific canine. In addition, lingual botton (3M Unitek Corporation, Monrovia, CA, USA) was placed on lingual surface of each canine and second premolar. The retraction forces, on average of 60 g, were applied through the elastic chains (Alastik™, 3M Unitek Coperation) linked between two retraction hooks on buccal side and also between two lingual bottons on lingual side. Each canine was retracted for 3 months. The patients were scheduled for every month. At each appointment during the experimental period, the elastic chains were replaced.

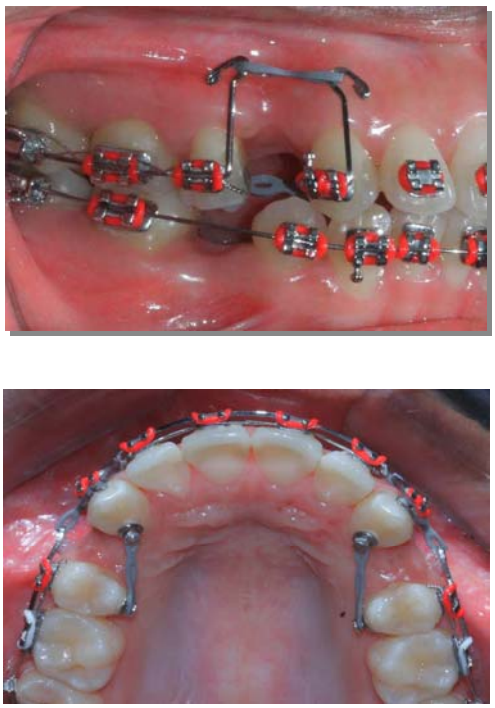

Fig.1: The mechanic for canine diatalization

Vibratory stimulation with electrical toothbrush

After the first month of canine retraction, right or left canine was randomly selected for the additional stimulation with electrical toothbrush (Colgate® Motion-Multi Action) The patients were informed to apply themselves the mechanical vibration by placing the electrical toothbrush on the labial surface of selected canine (Figure 2) as long as feasible per day for the last 2 months of experiment. The participants were assigned to report the duration of using electrical toothbrush and discontinue the vibration in the object of any inconvenience.

Fig.2: The demonstration of vibrating application via electrical toothbrush

The appointment schedule and data collection

A list of planned appointment for each patient is shown in Table 1. The GCF collection and upper impression taking for reference model were performed 5 times throughout the study.

Table 1: The appointment schedule and data collection

Visit	Treatment	Model & GCF
1 (baseline)	Initial record	√
2	bracket and wire placement	-
3 (T0)	start canine retraction	√
4 (T1)	1 month after canine retraction	√
5 (T2±V1)	2 months after canine retraction with or without 1-month vibration	√
6 (T3±V2)	3 months after canine retraction with or without 2-month vibration	√

GCF collection

GCF samples were collected from the mesiolabial and distolabial site of both left and right maxillary canines. Before GCF collection, the site was isolated with lip retractor. Any supragingival plaque was removed without invasion of gingival margin. The cervical area was gently dried with an air syringe.

The GCF was collected with No.30 standardized absorbent paper point (Hygenic®, Germany) which was inserted into the crevice at each site until mild resistance was felt and then left in place for 60 seconds while maintaining isolation as shown in Figure 3.

Fig.3: GCF collection from the gingival sulcus with No.30 standardized absorbent paper point.

The paper point visually contaminated with saliva or blood was discarded. The paper point was immediately transferred to 1.5 ml plastic tube. The absorbed GCF volume was quantified by the difference in weight of the paper point before and after GCF collection with digital weight measuring device (Sartorius MC210S). The paper point with GCF was snapped frozen in at –80°C until further processing was carried out.

Analysis of IL-1β level

GCF samples were assayed in triplicate to determine concentrations of IL-1β with commercially available IL-1β enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical, Ann Arbor, MI) as shown in Figure 4. In brief, 100 µl of diluted GCF in phosphate buffered saline (PBS) was added to each well of 96-well plate pre-coated with a monoclonal antibody specific for IL-1β followed with 100 µl of IL-1β (human) AChE Fab' Conjugate. Then covered the plate with plastic film and incubated overnight at 4^{o}C. The wells were emptied and rinsed five times with washing buffer. Ellman's reagent (200 µl) was added to each well. The plate was again covered and incubated on an orbital shaker at room temperature for 6 hours in the dark. The plates were read by using a PowerWave microplate reader (Biotex, England) at a wavelength of 412 nm. The concentration of IL-1β (pg/ml) was calculated from the GCF volume which considering GCF density value = 1.

GCF Preparation for ELISA assay

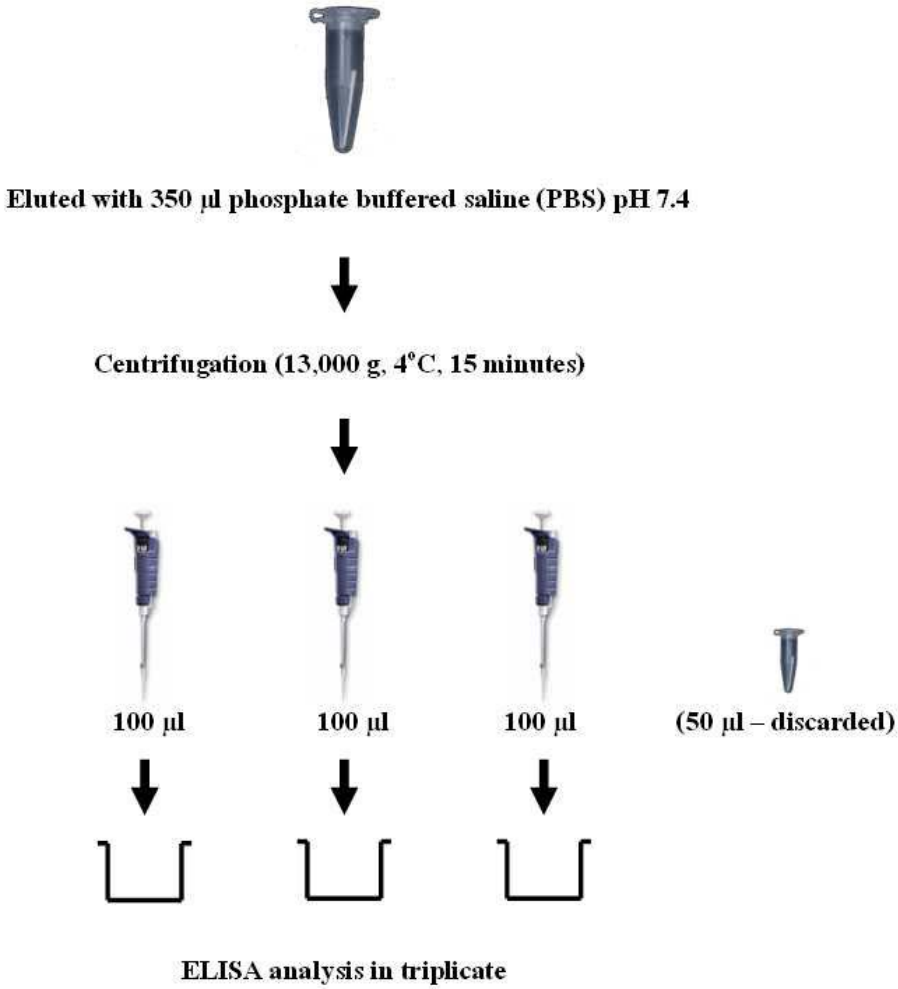

Fig.4: GCF preparation for ELISA assay. The –80°C store of GCF was eluted with 350 µl of PBS. After centrifugation at 4°C for 15 minutes, 100 µl aliquot part of elution was transferred to each well for ELISA analysis in triplicate.

Measurement of the rate of tooth movement

At each selected appointment, the impression taking of the maxillary arch was performed for stone model. The series of models from each subject were assessed the changes in canines' position relative to the stable landmark which was the ipsilateral median end of third rugae for each canine. The initial model was used for making of a palatal plug with reference wires for making measurement of canine movement as previously described by Limpanichkul. [8]

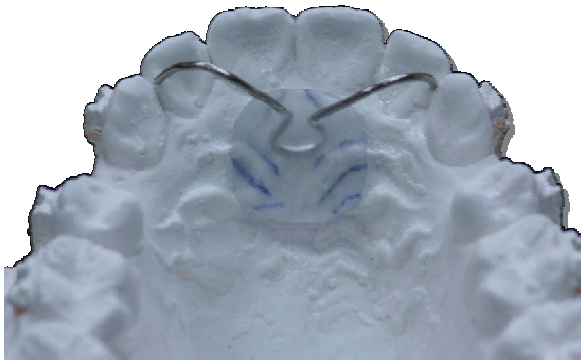

Fig.5: Acrylic plug for measuring the canine movement

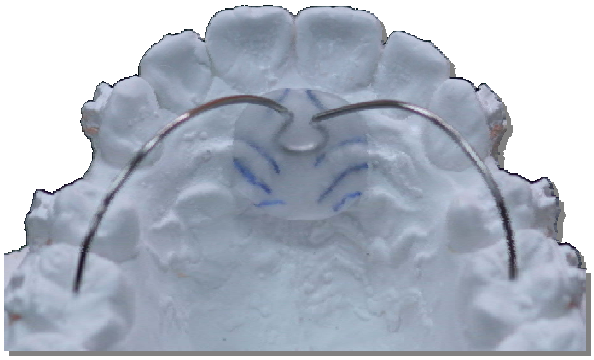

Fig.6: Acrylic plug for measuring movement of posterior units

Fabrication of acrylic palatal plug

An acrylic palatal plug was fabricated from clear orthodontic acrylic, covering just the medial portion of the palatal rugae with 0.9 mm stainless steel wire were set in an acrylic plug. For measuring the canine movement, the wire pointed to the most mesial surface of right and left canine as shown in Figure 5. For measuring anteroposterior movement of posterior units, the wire pointed to the central pit of right and left first molar as shown in Figure 6. The lines were marked on the exposed surface of the palatal plug coinciding with the incisive papilla point and the median raphe for additional landmarks while the plug was transferred to the progress models.

For measuring the canine movement, the distance between the wire end and the most mesial surface of each canine and also for the central pit of each first molar was measured with a digital caliper. All models were re-measured 2 weeks later. For assessing the rate of canine movement between with or without vibratory stimulation, the mean rate of canine movement between experiment and control side were compared among T1, T2$\pm$V1, and T3$\pm$V2. The technical error of measurement was evaluated by using paired t-test[63] which showed no statistical difference between the first and second time of measurement ($p<.05$).

Statistical analysis

Means and standard deviations of the rate of tooth movement, IL-1β level, and GCF volume were obtained for the experimental and control group at each time.

The Friedman test[64] was used to determined whether there were any significant overall differences in rate of tooth movement, IL-1β level, and GCF volume among the groups at each time. Wilcoxon Signed-Rank test[65] was performed to determine statistically significant differences in level of IL-1β, rate of tooth movement between, and GCF volume of experimental and control groups at each time.

CHAPTER 3

Results

In all participants, plaque accumulation was minimal throughout the study. No signs and symptoms of periodontal destruction were observed in any subject. There was no report in any discomfort from using electrical toothbrush.

Duration period for vibratory stimulation

The participants in this study were advocated to apply mechanical vibration with no strict limit on the amount of time. At the end of study, the record of electrical toothbrush using was returned form each participant. The patterns of electrical toothbrush usage were different among the subjects as reported in Table 2.

Table 2: The pattern of vibratory application from electrical toothbrush in each subject

Subject	Average duration per day (minute)	% day using toothbrush	frequency/day
1	4.8	100	1
2	17.8	100	1-2
3	18	100	1
4	19.2	100	1
5	20.2	86.2	1
6	30	100	1
7	31.9	71.9	1
8	42.3	100	2-3
9	45	65	1-2
10	52.6	100	6

The durations of using electrical toothbrush range from 4.8 to 52.6 minutes per day (mean 28.18 ± 14.90). Most subjects applied the vibratory stimulation once a day for everyday.

GCF volume

The means of collected GCF volume were shown in Table 3. The mean volume of GCF collected from both pressure (distal) and tension (mesial) sites of the control teeth were similar to that collected from the experimental teeth at baseline. The similarity mean volume of GCF between the control and experimental groups was also found at T0, T1, T2 ± V1, and T3 ± V2 as shown by Friedman test ($p<0.05$).

Table 3: GCF volume (mean ± SD) at pressure and tension sites from experimental and control groups (μl), calculated with the Friedman test ($p<0.05$).

	Pressure site (distal)		**Tension site (mesial)**		***P* value**
	control	**experiment**	**control**	**Experiment**	
Baseline	0.148 ± 0.060	0.128 ± 0.040	0.130 ± 0.060	0.143 ± 0.049	
T0	0.250 ± 0.114	0.271 ± 0.105	0.259 ± 0.118	0.237 ± 0.088	
T1	0.364 ± 0.096	0.387 ± 0.094	0.387 ± 0.109	0.376 ± 0.117	.733
T2±V1	0.240 ± 0.111	0.262 ± 0.109	0.311 ± 0.135	0.277 ± 0.091	
T3±V2	0.205 ± 0.058	0.167 ± 0.034	0.231 ± 0.131	0.182 ± 0.083	

The smallest GCF volume appeared at baseline which was slightly less than at the final observation period (T3 ± V2). The largest GCF volume was found after 1-month of orthodontic force application for canine retraction (T1).

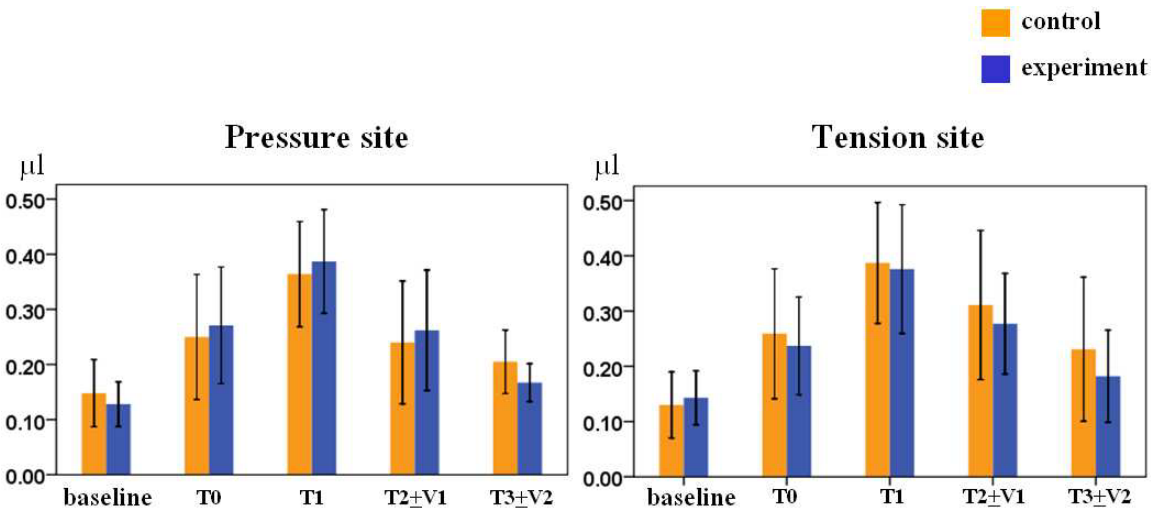

Fig.7: The fluctuation in GCF volume at each collected time

The fluctuating patterns of GCF volume were similar in both tension and pressure sites in both control and experimental groups as shown in Figure 7. The least GCF volume was found at the baseline, then increased almost double amount after bracketing for 1 month (T0). The increase continued to reach triple amount of the volume after the force was applied for 1 month (T1) which could be considered as the peak of GCF volume in this observation. After T1, the GCF volume started to reduce back toward the baseline. The GCF volumes at T2□V1 and T3□V2 were quite close to those at T1 and baseline respectively. The statistical differences between each time point were presented in Table 4 – 7.

Table 4: *P* value from the comparison of GCF volume between each time at pressure site of control group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2-V1	T3-V2
Baseline	.028*	.005**	.047*	.114
T0		.047*	.878	.262
T1			.013*	.005**
T2-V1				.476

Table 5: *P* value from the comparison of GCF volume between each time at tension site of control group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2-V1	T3-V2
Baseline	.046*	.005**	.009**	.012*
T0		.011*	.358	.385
T1			.203	.047*
T2-V1				.059

Table 6: *P* value from the comparison of GCF volume between each time at pressure site of experimental group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2□V1	T3□V2
Baseline	.007**	.005**	.012*	.007**
T0		.028*	.721	.014*
T1			.019*	.005**
T2□V1				.052

Table 7: *P* value from the comparison of GCF volume between each time at tension site of experimental group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2□V1	T3□V2
Baseline	.005**	.005**	.009**	.540
T0		.012*	.359	.240
T1			.046*	.005**
T2□V1				.028*

The rate of tooth movement

This study was designed to apply the force without vibratory stimuli in the first month of canine retraction at both control and experimental sites in order to allow the canine movement in the same amount at both sites. The result in Table 8 showed no significant difference in rate of canine movement between control and experimental sites at T1. The comparison between rate of tooth movement in control and experimental group showed that the rate of canine movement was significant higher in experimental group at T2–V1 and T3–V2 ($p<.05$).

Table 8: The distance (mean ± SD) of canine movement in the experimental and control group. Significance of differences between experiment & control in each month, calculated with the Wilcoxon signed-rank test * ($p<.05$)

	Rate of tooth movement (mm/month)		*P* value
	control	experiment	
T1	0.67 ± 0.25	0.68 ± 0.27	.959
T2±V1	0.85 ± 0.18	1.21 ± 0.27	.013*
T3±V2	0.62 ± 0.16	0.94 ± 0.20	.013*

In the control group, after continuing to perform canine retraction with the same force (T2-V1), the canine was moved with slightly faster rate. At T3-V2, The rate of tooth movement was slightly less than T2-V1. However, the Friedman test revealed no statistical significance among T1, T2-V1, and T3-V2 ($p<.05$).

In the experimental group, the rate was double elevated after continuing applying force together with 1-month vibration (T2–V1). In the second month of vibration (T3–V2), the rate was reduced but still faster than that of at the baseline (Figure 8).

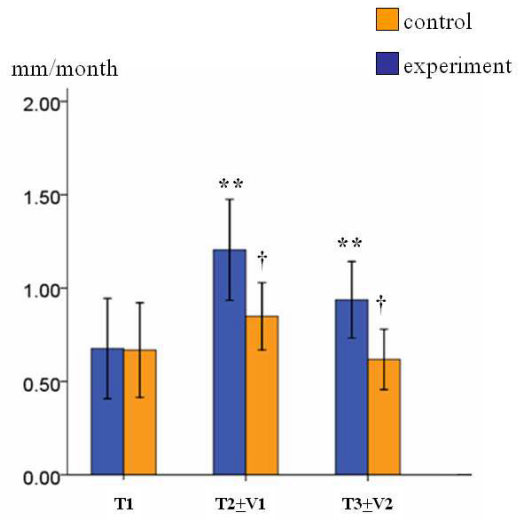

Fig.8: The rate of maxillary canine movement in the experimental and control groups

Significantly different from T1 in each month ** (p<.01)

Significantly different from experimental group at same time period † (p<.05)

Movement of posterior unit

The movement of posterior unit was found in this study. As shown in Figure 9, the mean SD of total movement of posterior unit during canine retraction for 3 months was 1.51 0.25 mm. in experimental group and 1.59 0.63 mm. in control group which no significant difference between two groups.

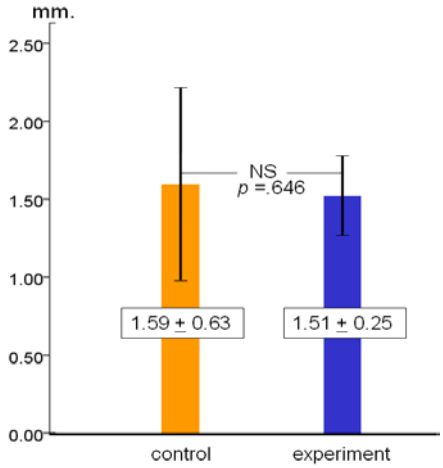

Fig.9: Total movement of posterior unit during the study. Error bars: - 1 SD

No statistical difference between control and experimental group (p<.05).

Although the amount of posterior unit movement in both groups were similar, the percentage of anchorage loss in control group (41.76%) was higher than experimental group (35.34%) as shown in Table 9.

Table 9: Percentage of anchorage loss

	control	experiment
Total space closure	3.79	4.27
Movement of posterior unit	1.59	1.51
Percentage of anchorage loss	41.76%	35.34%

IL-1β level

The IL-1β profile at pressure site of control group was shown in Figure 11. The IL-1β level was higher than baseline after canine retraction for 1 month (T1). Then the IL-1β level decreased to the baseline level after the canine was continue to retract in the second (T2-V1) and the third month (T3-V2).

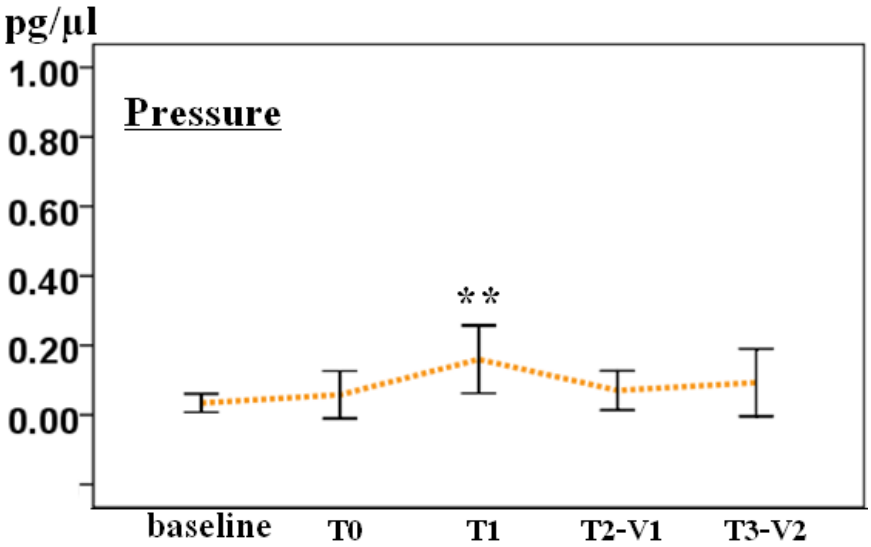

Fig. 10: IL-1β level between each time point at pressure site of control group

Significance of differences from baseline ** (p<.01) Error bars: +/- 1 SD

Table 10: *P* value from the comparison of IL-1β level between each time point at pressure site of control group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2-V1	T3-V2
Baseline	.721	.005**	.093	.285
T0		.005**	.508	.646
T1			.047*	.114
T2-V1				.575

The IL-1β profile at pressure site of experimental group was shown in Figure 12. Similar to the control group, the IL-1β level was higher than baseline after canine retraction for 1 month (T1). Interestingly, the IL-1β levels after that were quite distinct. At T2-V1, the result showed that the IL-1β level did not reduce but remained the same with T1. After the vibration was continually applied for another month (T3-V2), the -1β level was markedly elevate about 3 times of T2-V1.

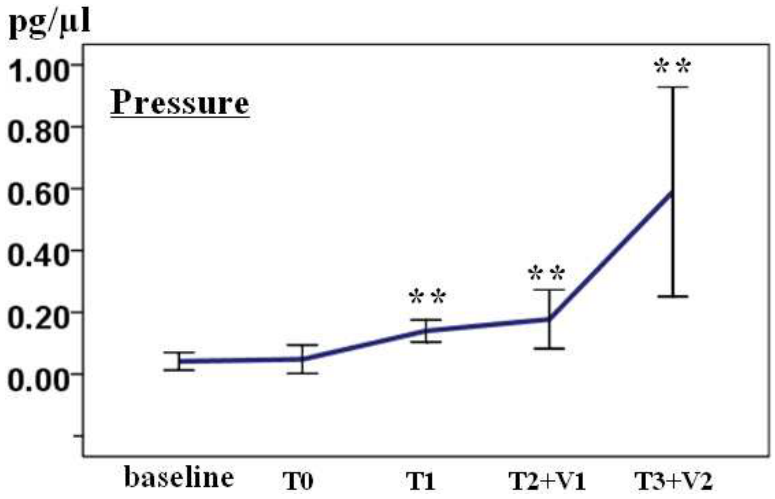

Fig. 11: IL-1β level between each time point at pressure site of experimental group Significance of differences from baseline ** (p<.01) Error bars: +/- 1 SD

Table 11: *P* value from the comparison of IL-1β level between each time point at pressure site of experimental group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2 – V1	T3 – V2
Baseline	.878	.005**	.005**	.005**
T0		.007**	.009**	.005**
T1			.575	.005**
T2 – V1				.007**

For the IL-1β level at tension site of control group, the fluctuation of IL-1β profile was unnoticed. The indifference among each time point was confirmed by Friedman test which demonstrated no significant difference among group (p<.05).

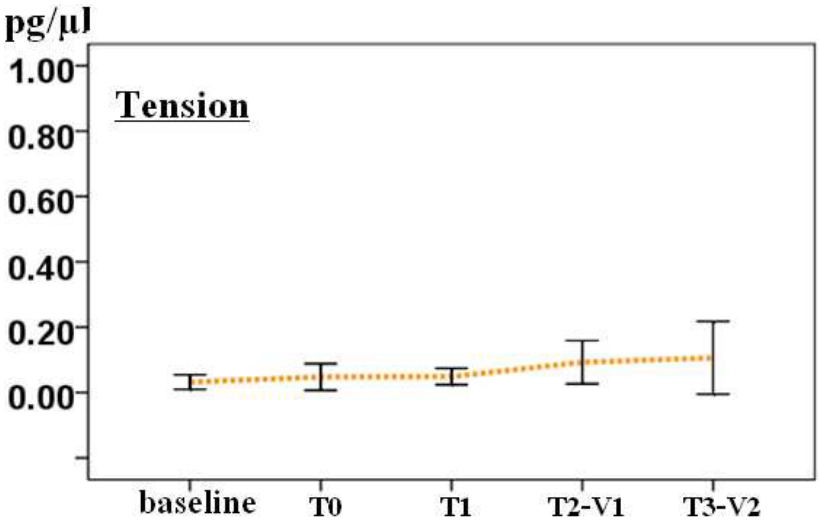

Fig. 12: IL-1β level between each time point at tension site of control group. Error bars: +/- 1SD

In similar to the control group, IL-1β profile of experimental group on tension site was insignificant changed from baseline to T1. But, the dissimilarity was seen after the vibratory stimulation for 1 month (T2 – V1) and 2 months (T3 – V2).

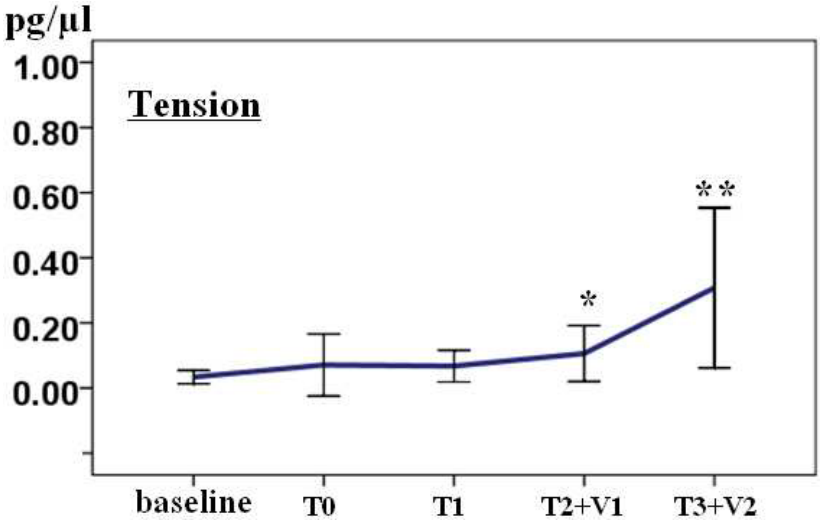

Fig. 13: IL-1β level between each time point at tension site of experimental group

Significance of differences from baseline * (p<.05) ** (p<.01) Error bars: +/- 1 SD

Table 12: P value from the comparison of IL-1β level between each time point at tension site of experimental group. Significance of differences between each month, calculated with Wilcoxon signed-rank test * (p<.05) ** (p<.01)

P value	T0	T1	T2_V1	T3_V2
Baseline	.241	.059	.028*	.005**
T0		.445	.333	.013*
T1			.285	.007**
T2_V1				.037*

The comparison between IL-1β profile of control and experimental group was shown in Figure 14, 15 and Table 13. At pressure site, the baseline level of IL-1β was indifference. The divergence between the IL-1β level from experimental and control group was seen after vibratory stimulation was combined with canine retraction force (T2_V1). The difference was markedly observed when the vibration was continually applied in experimental group for 2 months (T3_V2). For the tension site, the noticeable difference was found only at T3_V2.

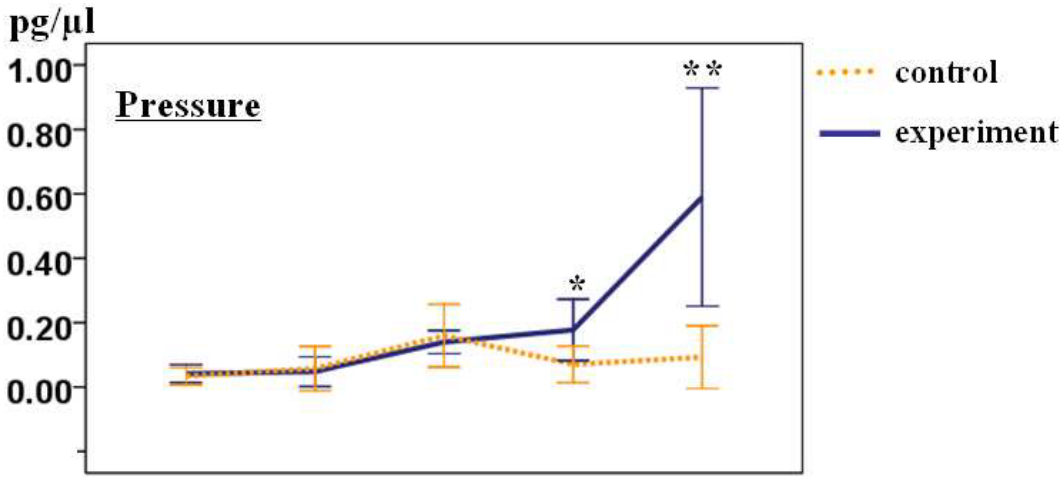

Fig.14: IL-1β profiles of control and experimental group at pressure site

Significance of differences from baseline * (p<.05) ** (p<.01) Error bars: ± 1 SD

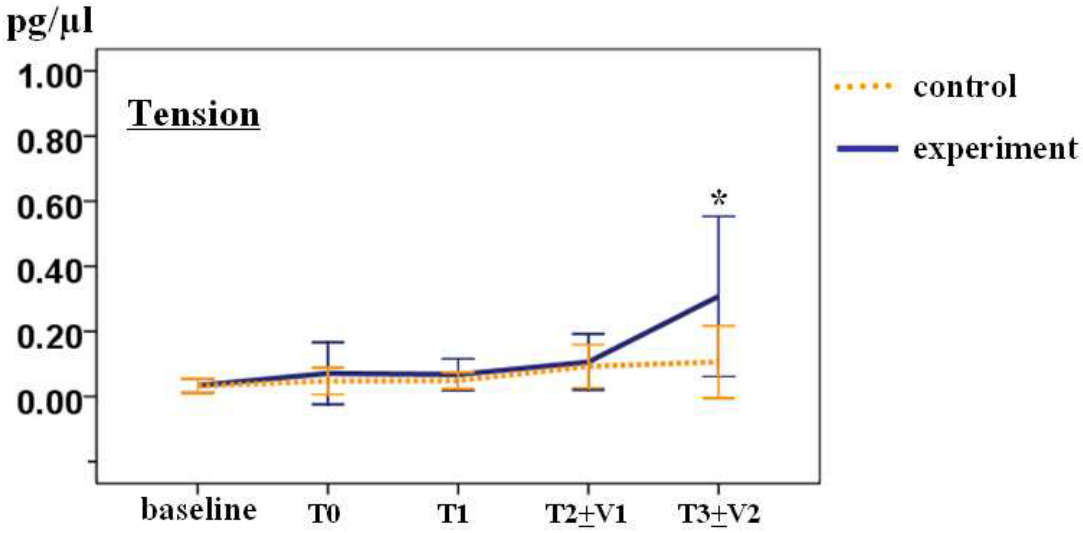

Fig.15: IL-1β profiles of control and experimental group at tension site

Significance of differences from baseline * (p<.05) Error bars: ± 1 SD

Table 13: The concentration (mean ± SD) of IL-1β from both experimental and control groups Significance of differences between experiment & control in each month, calculated with the Wilcoxon signed-rank test (p<.05)* (p<.01)**

	Control (pg/μl)	**Experiment** (pg/μl)	***P* value**
Pressure			
Baseline	0.034 ± 0.027	0.041 ± 0.029	.508
T0	0.058 ± 0.069	0.048 ± 0.046	.646
T1	0.160 ± 0.098	0.140 ± 0.036	.959
T2±V1	0.071 ± 0.056	0.177 ± 0.095	.013*
T3±V2	0.093 ± 0.097	0.590 ± 0.339	.005**
Tension			
Baseline	0.032 ± 0.022	0.034 ± 0.021	.799
T0	0.048 ± 0.041	0.071 ± 0.095	.878
T1	0.049 ± 0.025	0.068 ± 0.049	.575
T2±V1	0.093 ± 0.066	0.106 ± 0.086	.646
T3±V2	0.106 ± 0.111	0.308 ± 0.246	.022*

Assessment of the short term effect of vibratory stimulation (Supplementary study)

In order to clarify the effect of vibratory stimulation without orthodontic force on IL-1β secretion, eight volunteers (26 - 29 years old) with good oral health and no orthodontic appliances were involved in this study. Each subject was informed to apply themselves the mechanical vibration by gently placing the electrical toothbrush for 15 minutes per day on the labial surface of selected maxillary canine for 1 week of experimental period. The GCF was collected from the mesial site of canines in both experimental and control side at baseline and 1 week later for IL-1β analysis. With the Friedman test, the result revealed no significant difference in IL-1β level among 4 groups (p<.05).

Table 14: IL-1β level (mean ± SD) at initial time and 1-week after vibratory stimulation

	control (pg/μl)	**experiment** (pg/μl)
initial	0.041 ± 0.011	0.040 ± 0.009
1 week	0.042 ± 0.013	0.054 ± 0.015

CHAPTER 4

Discussion

Orthodontic mechanics

The aim of this study was to verify the possibility of vibratory stimulation in accelerating orthodontic tooth movement. In an initial plan, the mechanic for canine retraction was designed for sliding movement along the archwire by light force generated form NiTi closed coil spring as shown in Figure 16.

After clinical trials on 4 patients, the high friction between bracket and archwire presented the serious problems during the canine retraction. In Figure 17, the canine movements on both sides were small after retraction with NiTi closed coil springs (60 grams) for 3 months which might be represented too low magnitude of force. Therefore, the 60 grams of force was replaced with 90 grams for canine retraction.

Although, change in magnitude of force might be the solution for the rate of tooth movement, the problem of the existing friction was remained unresolved. The vibratory stimulation was failed to show the increase in rate of tooth movement. In order to eliminate the friction between bracket's slot and archwire, the particular design for canine retraction appliance was developed.

In sliding mechanics, the space between bracket slot and archwire afforded the initial canine tipping movement before bodily movement occurred during canine retraction which caused a large amount of friction. The stick-slip behavior of the bracket on the archwire may affected by tipping force and ligation. Iwasaki *et al.*[66] demonstrated the elimination of friction with vibration *in vitro* which showed faster bracket movement along the archwire after vibratory application. Although the clinical study of Iwasaki *et al.*[66] failed to demonstrate an effective reduction in bracket-archwire friction via vibration induced by mastication *in vivo* due to uncontrolled variations of intraoral environment and ligation techniques. The vibration was seemed to be affected to frictional system in sliding mechanic.

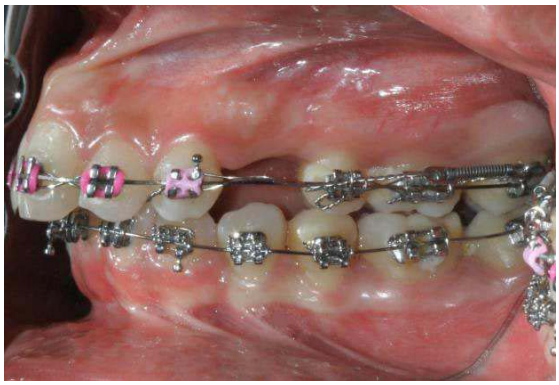

Fig.16: Preliminary design for canine retraction

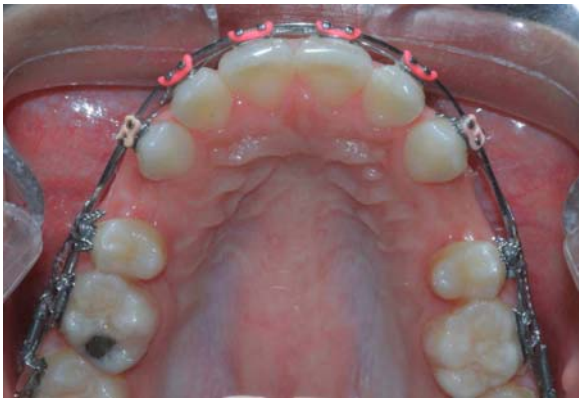

Fig.17: The distance of canine movement after retraction with 60 grams of force for 3 months. The picture showed small quantity in canine movement as the result of retraction with low magnitude of force.

In order to investigate the biological aspect of tissue reaction in canine acceleration with mechanical vibration, the design for inter-surface frictionless was strictly necessary. The wireless canine retraction however implicated undesirable crown tipping and rotation. A customized retraction hook and elastic chains on both sides were applied with low magnitude of force incorporated into the final design for canine retraction with attenuated side effects. Although distal tipping and disto-lingual rotation of retracted canine were unavoidable and did occur in both experimental and control groups, only small in amount of tipping and rotation were observed in some subjects (Figure 18 and 19).

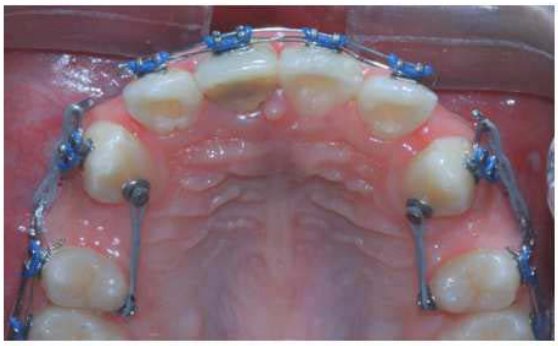

Fig.18: Disto-lingual rotation of retracted canine

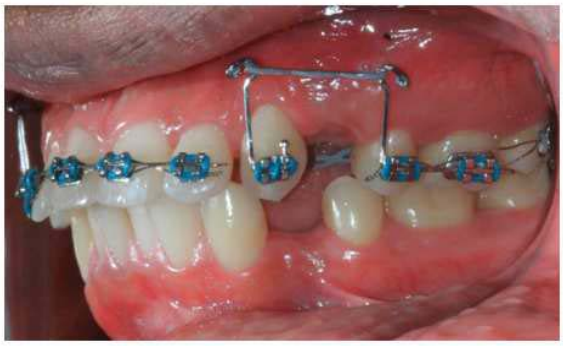

Fig.19: Distal tipping of retracted canine

The GCF volume

The fluctuations of GCF volume during orthodontic tooth movement and retention period are only partly due to changes in the severity of gingival inflammation.[67, 68] However, the GCF volume increased significantly during orthodontic treatment regardless the presence of inflammatory process in tissue reaction. In this study, the GCF volume elevated after 1-month retraction force application at T1. An initial canine movement might associate to the acute condition in periodontal tissue response. Similar to the study of Leethanakul *et al.*[69] which showed the highest volume of GCF within 1-month of canine retraction and had slightly declined in GCF volume at 2-month retraction. During the course of orthodontic treatment, the forces exerted produce a distortion of the periodontal ligament extracellular matrix, resulting in alterations in cellular shape and cytoskeletal configuration. Such events lead to the synthesis and

presence in the deeper periodontal tissues of extracellular matrix components, enzymes, and inflammatory mediators. These changes may modify both the GCF flow rate and its components.[70] The leakage of serum form periapical vessel during orthodontic tooth movement contributed to the high quantity of GCF in gingival sulcus in an early phase of inflammation. The quantity of GCF fluid may not represent the amount of total protein.[57] Although the IL-1β level in this study was increased according to vibratory stimulation, the deceleration of GCF volume was found during vibratory period. So, the vibratory stimulation in this study might not alter the GCF secretion. At T2±V1 and T3±V2, the GCF volume was decreased to baseline level while the tooth movement was increased. Therefore, the association between GCF volume and rate of tooth movement could not be found.

IL-1β profile and rate of tooth movement

In control group, the level of IL-1β at the pressure site was similar in fluctuating pattern from to the previous studies which showed the IL-1β level decreased to baseline level after one and two months of tooth movement.[69, 71, 72]

Changes in IL-1β profile of GCF form pressure site elicit the bone resorbing pattern due to direction of canine movement. The decrease in IL-1β level in T2-V1 and T3-V2 in comparative to T1 occurred as the normal activity in IL-1β secretion that mainly express in the early time of bone resorption while the rate of canine movement remained constant. A persistent tooth movement after IL-1β reduction was the result of downstream signaling cascade of bone resorbing mediators.[13]

In case of vibrating-combined orthodontic force, IL-1β level at the pressure site enhanced from T1 to T2+V1 in stead of depletion with in turn coincided to canine movement acceleration. This may reflect the possible function of more IL-1β secretion as a consequence of vibratory stimulation. IL-1β directly involves in bone resorption as a potent signaling molecule for RANKL expression of osteoblasts and human PDL cells and also directly stimulating the differentiation of osteoclast precursors.[62, 73]

Interestingly, after 2 months of vibratory stimulation (T3+V2), IL-1β level was not only maintained but also notably elevated about 3 times from T2+V1 while the rate of tooth

movement was reduced. This result dissociated to the studies of Iwasaki[2, 3] that showed positive correlation between IL-1β and rate of tooth movement.

The reason for this contrast was the dissimilarity between mechanical applications. The studies of Iwasaki presented no additional stimulation beside the conventional orthodontic force.

In this study, it might be possible that the inflammatory response has intensified in the last month of vibratory application. The inflammatory response is mediated mainly by tissue-resident and recruited immune cells. Macrophages are the potent cells in adaptive immunity which are migrated to PDL area in later stage of orthodontic tooth movement and have the ability to produce IL-1β.[74] So, the secretion of this inflammatory cytokine from macrophages might result in marked elevation of IL-1β level in experimental group which were not involved with rate of tooth movement.

Another rationale behind the dissociation between extra secretion of IL-1β and rate of tooth movement can be explained in perspective on the homeostatic function of PDL fibroblasts via osteoprotegerin (OPG). Osteoclastogenesis is primarily activated by RANKL and inhibited by OPG. The experimental tooth movement in rats with local OPG gene transfer at the compressive site of the periodontium could neutralize the RANKL activity induced by the mechanical force, inhibiting osteoclastogenesis and diminishing orthodontic tooth movement.[75] As mention earlier that PDL fibroblasts may have dual effects on capable of inducing the formation of osteoclast-like cells and also inhibiting them according to the conditions examined. Kook Sung-Ho e*t al.* [41] reported that centrifugal force inhibited osteoclastogenesis of the PDL fibroblasts through OPG production.

Another in vitro study by Kansaki *et al.*[39] demonstrated the inhibition of the osteoclastogenesis-supporting activity of PDL cells by inducing the up-regulation of OPG under cyclical tensile-force stimulation. In addition, PDL cells under intermittent tensile stress not only up-regulate OPG mRNA expression but also tissue inhibitor of matrix metalloprotease-1, 2 which inhibit the extracellular matrix protein degradating activity of matrix metalloproteases. [76] Furthermore, some studies showed that mechanical vibration was able to partially inhibit the bone resorption and enhance anabolic activity of bone.[20, 22, 77-79]

In case of osteoblasts and osteocytes, there are sufficient evidences to justify their roles in bone remodeling process under the vibratory stimuli. Many studies indicate their

anabolic activity in bone formation. Osteoblasts and osteocytes are able to sense low-magnitude, high-frequency vibration and respond by increase in production of OPG or decrease in RANKL expression which caused the inhibition of osteoclast formation.[18-22, 33-35, 37, 79-82]

Mechanosensing by several cell types in bone and surrounding tissues in response to the challenges of frequency mechanical loading is unknown and difficult to clarify. It has been seemed that the vibratory stimulation and bone activity is controversial. Especially for the dentoalveolar complex which the mainly role of bone homeostasis is under the equilibrium control of PDL fibroblasts and bone cells. This study might not introduce any particular function of any cells that get involved. Change in rate of tooth movement and IL-1β level in this study only represented the experimental outcome as the results of biological summary from the clinical approach. Although the elevation of IL-1β level has a relationship with the severity of periodontal diseases, the maximum concentration of IL-1β that presented in this study at T2+V1 and T3+V2 were 0.38 pg/µl and 1.09 pg/µl respectively which were marked below the IL-1β level in periodontitis (5.4 $\pm$ 4.07 pg/µl) and gingivitis (2.0 $\pm$ 2.49 pg/µl).[83] So, an increase in IL-1β level in this study demonstrated within the physiological response with no clinical complication. Anyway, an accumulation of IL-1β after continuous mechanical vibration over 2 months of canine retraction is possible and that should be highly considered before the vibratory stimulation is supposed to be an intervention on the routine practice.

For the tension site, the gradually increase of IL-1β level from baseline represented the small incremental proceed in bone resorption occurred during initial wire placement and more extend in retraction phase. A slight root tipping could happen while the canine was retracted which caused the bone resorption in mesial site. The marked elevation of IL-1β level at T3+V2 when compare to T3-V2 might be the result of accumulative vibratory exposure.

Although the marked elevation of IL-1β was demonstrated in vibratory group, the significant increase in IL-1β level after 1-week of vibration in non-orthodontic subjects was not found in this study. The elevation of IL-1β due to the combination of vibration and orthodontic force may elicit in synergistic manner but not in accumulation pattern. Nevertheless, the slightly increase in IL-1β level was noticed. So, the non significance in IL-1β level of supplementary study may not represent too short period for vibratory stimulation. The further study is required for this issue.

The duration of using electrical toothbrush

The occurrence of adverse effects from vibration was reported in several studies of occupational vibration-exposure. In human study,[84] the decrease in blood flow was found after short-term exposure to 125-Hz vibration as the result of temporary vasoconstriction while the skin temperature and systolic blood pressure did not change significantly during vibration exposure for 30 minutes. In contrast, prolonged exposure to hand-transmitted vibration can cause permanent debilitating neural and vascular dysfunction in workers who exposed to high magnitude of vibration for several hours daily.[85, 86] In this study, the 50 minutes of vibratory duration might cause a transient adverse effect with no any irreversible damage.

<The duplication from Table 2>

Subject	Average duration per day (minute)	% day using toothbrush	frequency/day
1	***4.8***	***100***	***1***
2	17.8	100	1-2
3	18	100	1
4	19.2	100	1
5	20.2	86.2	1
6	30	100	1
7	31.9	71.9	1
8	***42.3***	***100***	***2-3***
9	45	65	1-2
10	52.6	100	6

With Pearson correlation analysis, the relationship between vibrating duration and IL-1β level can not be found in this study. Interestingly, the non-significant difference in rate of tooth movement between experimental and control sites were observed in the subjects No.1 and No.8. The minimum of stimulating duration (4.8 minute/day) was shown in No.1 which might represent an insufficient period of vibratory stimulation.

The IL-1β level in this case also displayed no difference between experimental and control sites. In case of No.8, this subject applied the mechanical vibration for a long time per day (42.3 minute/day). This might not be the greatest time among the subjects. However, this subject used electrical toothbrush with no resting day and less resting period during the day which compared to No.9 and No.10 respectively. The previous study in animal model showed that insertion of rest periods between high-frequency stimuli significantly augmented the osteogenic activities.[87] Therefore, resting period may require for too long stimulatory period in this study.

To mention an idea for the use of vibratory stimulation on tooth movement acceleration for the future study to consider, short duration with the insertion of rest periods during the loading phase is suggestive of vibrating approach.

The magnitude and frequency of vibration

Low level of magnitude with high frequency of vibration is subjected to enhance the efficacy of the mechanical-induced bone activities.[18, 22, 35] According to the frequency used in the literatures, the 125 Hz of vibration from electrical toothbrush was accepted as broad-frequency vibration. Although the participants in this study were advised to gently put the brush on the tooth surface, the magnitude of mechanical vibration might not be identified. The muscle fatigue resulted from prolonged holding of toothbrush might alter the magnitude of force applied during the vibratory stimulation. Hand preference might has an influence on the variation of magnitude among the subjects because the motor fibers of muscle on the dominant side of hand are greater in firing rate and force delay which showed the mechanical effectiveness of motor function.[88] However, this study could not demonstrate any remarkable information about the handedness of electrical toothbrush usage.

Limitation of the study

There is no particular cytokine that can be the sole factor in biomarker that explains the process of bone remodeling.[89] The resultant IL-1β level measured in the gingival

crevicular fluid is the sum of the various cellular responses to the pressure experienced at various locations on the pressure side. This is considered to be the limitation of *in vivo* experiment that must be overcome.

As mentioned earlier, the osteocytes are hypothesized to recognize this additional stimulation and activate bone tissue remodeling. The vibration, somehow, can be the external stimulation which optimize the signal transduction and induce the expression of essence chemical mediators for alveolar bone turnover. The gingival sulcus was selected as the testing site because of its continuity with the PDL and its accessibility within the oral cavity. The regulatory molecule in GCF can be appropriately used to identify the tissue response without damaging the periodontium. However, this convenient method brings about the limitation of data interpretation. GCF is composed of many cells and extracellular secretion from the tissue nearby. It is difficult to define the specific type of cells that take a responsibility for any change in GCF profile. Especially for the osteocytes, the buried bone cells, which their functions are barely detected in living person. PDL cells and osteoblasts are also the key regulation of osteoclast formation and function in orthodontic tooth movement. This clinical study might not provide the definite explanation for the complexity of cell-cell interactions in periodontium that occurred in this experiment. The efficient period of vibratory stimulation for accelerate tooth movement is hardly to identify. The stimulatory period in this study depended on the feasibility of each participant without the researcher dictating for the reason of willing to use and actual record. The previous studies in rat may not provide any guideline for practical timing in human. From this study, the vibratory stimulation could be an alternative method for further research in tooth movement acceleration. The long term clinical study with proper monitoring on periodontal status and anchorage situation are required before the clinical application. For the future study, the animal study and other biomarkers for bone formation may clarify the biological events and the efficient period of vibratory stimulation.

CHAPTER 5

Conclusion

With a simple instrument, the electrical toothbrush can provide a mechanical stimulation that is able to accelerate orthodontic tooth movement. IL-1β expression is found during orthodontic tooth movement and can be measured with crevicular fluid analysis. In combination with the orthodontic force, the vibratory stimulation does enhance IL-1β secretion in the gingival crevicular fluid. Which represent the up-regulation in activity of bone resorption. With the mechanical vibration alone, marked increase in IL-1β level may not be observed. Fluctuation of GCF volume is found with no correlation to the rate of tooth movement and IL-1β profile.

Any pathological condition is not present in mechanical vibratory application in single tooth for a period of time.

REFERENCES

1. Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. ***J Dent Res*** 2009; 88: 597-608.
2. Iwasaki LR, Chandler JR, Marx DB, Pandey JP, Nickel JC. IL-1 gene polymorphisms, secretion in gingival crevicular fluid, and speed of human orthodontic tooth movement. ***Orthod Craniofac Res*** 2009; 12: 129-40.
3. Iwasaki LR, Gibson CS, Crouch LD, Marx DB, Pandey JP, Nickel JC. Speed of tooth movement is related to stress and IL-1 gene polymorphisms. ***Am J Orthod Dentofacial Orthop*** 2006; 130: 698 e1-9.
4. Spielmann T, Wieslander L, Hefti AF. [Acceleration of orthodontically induced tooth movement through the local application of prostaglandin (PGE1)]. ***Schweiz Monatsschr Zahnmed*** 1989; 99: 162-5.
5. Yamasaki K, Shibata Y, Imai S, Tani Y, Shibasaki Y, Fukuhara T. Clinical application of prostaglandin E1 (PGE1) upon orthodontic tooth movement. ***Am J Orthod*** 1984; 85: 508-18.
6. Davidovitch Z, Finkelson MD, Steigman S, Shanfeld JL, Montgomery PC, Korostoff E. Electric currents, bone remodeling, and orthodontic tooth movement. II. Increase in rate of tooth movement and periodontal cyclic nucleotide levels by combined force and electric current. ***Am J Orthod*** 1980; 77: 33-47.
7. Hashimoto H. Effect of micro-pulsed electricity on experimental tooth movement. ***Nippon Kyosei Shika Gakkai Zasshi*** 1990; 49: 352-61.
8. Limpanichkul W, Godfrey K, Srisuk N, Rattanayatikul C. Effects of low-level laser therapy on the rate of orthodontic tooth movement. ***Orthod Craniofac Res*** 2006; 9: 38-43.
9. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low-level laser therapy during orthodontic movement: a preliminary study. ***Lasers Med Sci*** 2008; 23: 27-33.
10. Iseri H, Kisnisci R, Bzizi N, Tuz H. Rapid canine retraction and orthodontic treatment with dentoalveolar distraction osteogenesis. ***Am J Orthod Dentofacial Orthop*** 2005; 127: 533-41; quiz 625.

11. Sukurica Y, Karaman A, Gurel HG, Dolanmaz D. Rapid canine distalization through segmental alveolar distraction osteogenesis. ***Angle Orthod*** 2007; 77: 226-36.
12. Hermanson PC. Alveolar bone remodeling incident to tooth movement. ***Angle Orthod*** 1972; 42: 107-15.
13. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. ***Am J Orthod Dentofacial Orthop*** 2006; 129: 469 e1-32.
14. Burstone CJ. Variable-modulus orthodontics. ***Am J Orthod*** 1981; 80: 1-16.
15. Andreasen G, Johnson P. Experimental findings on tooth movements under two conditions of applied force. ***Angle Orthod*** 1967; 37: 9-12.
16. Hixon EH, Atikian H, Callow GE, McDonald HW, Tacy RJ. Optimal force, differential force, and anchorage. ***Am J Orthod*** 1969; 55: 437-57.
17. Owman-Moll P, Kurol J, Lundgren D. The effects of a four-fold increased orthodontic force magnitude on tooth movement and root resorptions. An intra-individual study in adolescents. ***Eur J Orthod*** 1996; 18: 287-94.
18. Castillo AB, Alam I, Tanaka SM, Levenda J, Li J, Warden SJ, et al. Low-amplitude, broad-frequency vibration effects on cortical bone formation in mice. ***Bone*** 2006; 39: 1087-96.
19. Garman R, Gaudette G, Donahue LR, Rubin C, Judex S. Low-level accelerations applied in the absence of weight bearing can enhance trabecular bone formation. ***J Orthop Res*** 2007; 25: 732-40.
20. Judex S, Lei X, Han D, Rubin C. Low-magnitude mechanical signals that stimulate bone formation in the ovariectomized rat are dependent on the applied frequency but not on the strain magnitude. ***J Biomech*** 2007; 40: 1333-9.
21. Ozcivici E, Garman R, Judex S. High-frequency oscillatory motions enhance the simulated mechanical properties of non-weight bearing trabecular bone. ***J Biomech*** 2007; 40: 3404-11.
22. Xie L, Jacobson JM, Choi ES, Busa B, Donahue LR, Miller LM, et al. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. ***Bone*** 2006; 39: 1059-66.
23. Nishimura M, Chiba M, Ohashi T, Sato M, Shimizu Y, Igarashi K, et al. Periodontal tissue activation by vibration: intermittent stimulation by resonance vibration accelerates experimental tooth movement in rats. ***Am J Orthod Dentofacial Orthop*** 2008; 133: 572-83.

24. Bonewald LF. Osteocytes: a proposed multifunctional bone cell. ***J Musculoskelet Neuronal Interact*** 2002; 2: 239-41.
25. Cowin SC. Mechanosensation and fluid transport in living bone. ***J Musculoskelet Neuronal Interact*** 2002; 2: 256-60.
26. Duncan RL, Turner CH. Mechanotransduction and the functional response of bone to mechanical strain. ***Calcif Tissue Int*** 1995; 57: 344-58.
27. Nicolella DP, Moravits DE, Gale AM, Bonewald LF, Lankford J. Osteocyte lacunae tissue strain in cortical bone. ***J Biomech*** 2006; 39: 1735-43.
28. Ren Y, Maltha JC, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a systematic literature review. ***Angle Orthod*** 2003; 73: 86-92.
29. Ren Y, Maltha JC, Van 't Hof MA, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a mathematic model. ***Am J Orthod Dentofacial Orthop*** 2004; 125: 71-7.
30. Burger EH, Klein-Nulend J. Mechanotransduction in bone--role of the lacuno-canalicular network. ***FASEB J*** 1999; 13 Suppl: S101-12.
31. Noble BS. The osteocyte lineage. ***Arch Biochem Biophys*** 2008; 473: 106-11.
32. Huang RP, Rubin CT, McLeod KJ. Changes in postural muscle dynamics as a function of age. ***J Gerontol A Biol Sci Med Sci*** 1999; 54: B352-7.
33. Rubin C, Turner AS, Bain S, Mallinckrodt C, McLeod K. Anabolism. Low mechanical signals strengthen long bones. ***Nature*** 2001; 412: 603-4.
34. Rubin C, Turner AS, Muller R, Mittra E, McLeod K, Lin W, et al. Quantity and quality of trabecular bone in the femur are enhanced by a strongly anabolic, noninvasive mechanical intervention. ***J Bone Miner Res*** 2002; 17: 349-57.
35. Tanaka SM, Li J, Duncan RL, Yokota H, Burr DB, Turner CH. Effects of broad frequency vibration on cultured osteoblasts. ***J Biomech*** 2003; 36: 73-80.
36. Eisman JA. Good, good, good... good vibrations: the best option for better bones? ***Lancet*** 2001; 358: 1924-5.
37. Gilsanz V, Wren TA, Sanchez M, Dorey F, Judex S, Rubin C. Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. ***J Bone Miner Res*** 2006; 21: 1464-74.

38. Middleton J, Jones M, Wilson A. The role of the periodontal ligament in bone modeling: the initial development of a time-dependent finite element model. ***Am J Orthod Dentofacial Orthop*** 1996; 109: 155-62.
39. Kanzaki H, Chiba M, Sato A, Miyagawa A, Arai K, Nukatsuka S, et al. Cyclical tensile force on periodontal ligament cells inhibits osteoclastogenesis through OPG induction. ***J Dent Res*** 2006; 85: 457-62.
40. Kanzaki H, Chiba M, Shimizu Y, Mitani H. Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor kappaB ligand up-regulation via prostaglandin E2 synthesis. ***J Bone Miner Res*** 2002; 17: 210-20.
41. Kook SH, Son YO, Hwang JM, Kim EM, Lee CB, Jeon YM, et al. Mechanical force inhibits osteoclastogenic potential of human periodontal ligament fibroblasts through OPG production and ERK-mediated signaling. ***J Cell Biochem*** 2009; 106: 1010-9.
42. Nakao K, Goto T, Gunjigake KK, Konoo T, Kobayashi S, Yamaguchi K. Intermittent force induces high RANKL expression in human periodontal ligament cells. ***J Dent Res*** 2007; 86: 623-8.
43. Emata T. The mechanical response of the periodontal structure in the maxillary lateral incisor of the macaca fuscata yakui, loading by a vibrating force. ***J Oral Biol Sci***. [journal]. 1979; 21: 571-85.
44. Ohmae M, Saito S, Morohashi T, H Q, Seki K, Kurabayashi H. Biomechanical acceleration of experimental tooth movement by ultrasonic vibration in vivo—part 1. Homo-directional application of ultrasonication to orthodontic force. ***Orthod Waves*** 2001; 60: 201-12.
45. Krasse B. Serendipity or luck: stumbling on gingival crevicular fluid. ***J Dent Res*** 1996; 75: 1627-30.
46. Goodson JM. Gingival crevice fluid flow. ***Periodontol 2000*** 2003; 31: 43-54.
47. Prapulla DV, Sujatha PB, Pradeep AR. Gingival crevicular fluid VEGF levels in periodontal health and disease. ***J Periodontol*** 2007; 78: 1783-7.
48. Griffiths GS. Formation, collection and significance of gingival crevice fluid. ***Periodontol 2000*** 2003; 31: 32-42.
49. Curtis MA, Griffiths GS, Price SJ, Coulthurst SK, Johnson NW. The total protein concentration of gingival crevicular fluid. Variation with sampling time and gingival

inflammation. ***J Clin Periodontol*** 1988; 15: 628-32.

50. Mundy GR. Cytokines and local factors which affect osteoclast function. ***Int J Cell Cloning*** 1992; 10: 215-22.
51. Nakashima T, Takayanagi H. Osteoclasts and the immune system. ***J Bone Miner Metab*** 2009; 27: 519-29.
52. Meager A. Cytokine regulation of cellular adhesion molecule expression in inflammation. ***Cytokine Growth Factor Rev*** 1999; 10: 27-39.
53. Wu CC, Li YS, Haga JH, Wang N, Lian IY, Su FC, et al. Roles of MAP kinases in the regulation of bone matrix gene expressions in human osteoblasts by oscillatory fluid flow. ***J Cell Biochem*** 2006; 98: 632-41.
54. Cowin SC, Weinbaum S. Strain amplification in the bone mechanosensory system. ***Am J Med Sci*** 1998; 316: 184-8.
55. Jendrucko RJ, Hyman WA, Newell PH, Jr., Chakraborty BK. Theoretical evidence for the generation of high pressure in bone cells. ***J Biomech*** 1976; 9: 87-91.
56. Ren Y, Hazemeijer H, de Haan B, Qu N, de Vos P. Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. ***J Periodontol*** 2007; 78: 453-8.
57. Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. ***Eur J Oral Sci*** 2008; 116: 89-97.
58. 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.
59. Dinarello CA. Interleukin-1. ***Cytokine Growth Factor Rev*** 1997; 8: 253-65.
60. Lee KJ, Park YC, Yu HS, Choi SH, Yoo YJ. Effects of continuous and interrupted orthodontic force on interleukin-1beta and prostaglandin E2 production in gingival crevicular fluid. ***Am J Orthod Dentofacial Orthop*** 2004; 125: 168-77.
61. Shimizu N, Yamaguchi M, Goseki T, Ozawa Y, Saito K, Takiguchi H, et al. Cyclic-tension force stimulates interleukin-1 beta production by human periodontal ligament cells. ***J Periodontal Res*** 1994; 29: 328-33.
62. Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced

osteoclastogenesis. ***J Clin Invest*** 2005; 115: 282-90.

63. Harris EF, Smith RN. Accounting for measurement error: A critical but often overlooked process. ***Archives of Oral Biology*** 2009; 54: S107-S17.
64. Rahlfs VW, Zimmermann H. Nonparametric methods in standard statistics packages: The case of the Friedman analysis. ***Computational Statistics & Data Analysis*** 1995; 20: 101-10.
65. Dewan I, Rao BLSP. Wilcoxon-signed rank test for associated sequences. ***Statistics & Probability Letters*** 2005; 71: 131-42.
66. Iwasaki LR, Beatty MW, Randall CJ, Nickel JC. Clinical ligation forces and intraoral friction during sliding on a stainless steel archwire. ***Am J Orthod Dentofacial Orthop*** 2003; 123: 408-15.
67. Samuels RH, Pender N, Last KS. The effects of orthodontic tooth movement on the glycosaminoglycan components of gingival crevicular fluid. ***J Clin Periodontol*** 1993; 20: 371-7.
68. Pender N, Samuels RH, Last KS. The monitoring of orthodontic tooth movement over a 2-year period by analysis of gingival crevicular fluid. ***Eur J Orthod*** 1994; 16: 511-20.
69. Leethanakul C, Kittichaikarn C, charoemratrote C, Jitpukdeebodintra S. Effects of Continuous and Interrupted Orthodontic Force on Interleukin1-beta and Interleukin-8 Secretion in Human Gingival crevicular fluid. ***J Oral Biosci*** 2008; 50: 230-8.
70. Kavadia-Tsatala S, Kaklamanos EG, Tsalikis L. Effects of orthodontic treatment on gingival crevicular fluid flow rate and composition: clinical implications and applications. ***Int J Adult Orthodon Orthognath Surg*** 2002; 17: 191-205.
71. Dudic A, Kiliaridis S, Mombelli A, Giannopoulou C. Composition changes in gingival crevicular fluid during orthodontic tooth movement: comparisons between tension and compression sides. ***Eur J Oral Sci*** 2006; 114: 416-22.
72. Yamaguchi M, Yoshii M, Kasai K. Relationship between substance P and interleukin-1beta in gingival crevicular fluid during orthodontic tooth movement in adults. ***Eur J Orthod*** 2006; 28: 241-6.
73. Nukaga J, Kobayashi M, Shinki T, Song H, Takada T, Takiguchi T, et al. Regulatory effects of interleukin-1beta and prostaglandin E2 on expression of receptor activator of nuclear factor-kappaB ligand in human periodontal ligament cells. ***J Periodontol*** 2004; 75: 249-59.

74. Vandevska-Radunovic V, Kvinnsland IH, Kvinnsland S, Jonsson R. Immunocompetent cells in rat periodontal ligament and their recruitment incident to experimental orthodontic tooth movement. ***Eur J Oral Sci*** 1997; 105: 36-44.

75. Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H. Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement. ***J Dent Res*** 2004; 83: 920-5.

76. Tsuji K, Uno K, Zhang GX, Tamura M. Periodontal ligament cells under intermittent tensile stress regulate mRNA expression of osteoprotegerin and tissue inhibitor of matrix metalloprotease-1 and -2. ***J Bone Miner Metab*** 2004; 22: 94-103.

77. Christiansen BA, Bayly PV, Silva MJ. Constrained tibial vibration in mice: a method for studying the effects of vibrational loading of bone. ***J Biomech Eng*** 2008; 130: 044502.

78. de Oliveira ML, Bergamaschi CT, Silva OL, Nonaka KO, Wang CC, Carvalho AB, et al. Mechanical vibration preserves bone structure in rats treated with glucocorticoids. ***Bone***.

79. Lynch MA, Brodt MD, Silva MJ. Skeletal effects of whole-body vibration in adult and aged mice. ***J Orthop Res*** 2009; 28: 241-7.

80. Lau E, Al-Dujaili S, Guenther A, Liu D, Wang L, You L. Effect of low-magnitude, high-frequency vibration on osteocytes in the regulation of osteoclasts. ***Bone*** 2010.

81. Tan SD, de Vries TJ, Kuijpers-Jagtman AM, Semeins CM, Everts V, Klein-Nulend J. Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. ***Bone*** 2007; 41: 745-51.

82. Vezeridis PS, Semeins CM, Chen Q, Klein-Nulend J. Osteocytes subjected to pulsating fluid flow regulate osteoblast proliferation and differentiation. ***Biochem Biophys Res Commun*** 2006; 348: 1082-8.

83. Figueredo CM, Ribeiro MS, Fischer RG, Gustafsson A. Increased interleukin-1beta concentration in gingival crevicular fluid as a characteristic of periodontitis. ***J Periodontol*** 1999; 70: 1457-63.

84. Bovenzi M, Lindsell CJ, Griffin MJ. Duration of acute exposures to vibration and finger circulation. ***Scand J Work Environ Health*** 1998; 24: 130-7.

85. Govindaraju SR, Curry BD, Bain JL, Riley DA. Effects of temperature on vibration-induced damage in nerves and arteries. ***Muscle Nerve*** 2006; 33: 415-23.

86. Necking LE, Lundborg G, Lundstrom R, Thornell LE, Friden J. Hand muscle pathology after long-term vibration exposure. ***J Hand Surg Br*** 2004; 29: 431-7.

87. LaMothe JM, Zernicke RF. Rest insertion combined with high-frequency loading enhances osteogenesis. ***J Appl Physiol*** 2004; 96: 1788-93.

88. Adam A, De Luca CJ, Erim Z. Hand dominance and motor unit firing behavior. ***J Neurophysiol*** 1998; 80: 1373-82.

89. Teitelbaum SL. Bone resorption by osteoclasts. ***Science*** 2000; 289: 1504-8.

APPENDICES

เอกสารชี้แจงและยินยอมเข้าร่วมการวิจัย

ในเอกสารนี้อาจมีข้อความที่ท่านอ่านแล้วยังไม่เข้าใจ โปรดสอบถามผู้วิจัยหรือผู้แทนให้ช่วยอธิบายจนกว่าจะเข้าใจดี ท่านอาจจะขอเอกสารนี้กลับไปอ่านที่บ้านเพื่อปรึกษาหารือกับญาติพี่น้อง เพื่อนสนิท แพทย์ประจำตัวของท่าน หรือแพทย์ท่านอื่น เพื่อช่วยในการตัดสินใจเข้าร่วมการวิจัย

ชื่อโครงการ การเปลี่ยนแปลงอัตราเร็วในการเคลื่อนฟันและระดับอินเตอร์ลูคิน 1 ชนิดเบต้าในน้ำเหลืองเหงือกระหว่างการเคลื่อนฟันทางทันตกรรมจัดฟันร่วมกับการใช้แรงสั่นสะเทือนจากแปรงสีฟันไฟฟ้า

ชื่อผู้วิจัย นาย สุมิตร สูอำพัน
นักศึกษาหลังปริญญาสาขาวิชาวิทยาศาสตร์สุขภาพช่องปาก (ทันตกรรมจัดฟัน)
ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

โครงการวิจัยนี้ทำขึ้นเพื่อศึกษาทางเลือกใหม่ที่จะช่วยลดระยะเวลาในการรักษาทางทันตกรรม จัดฟัน ซึ่งจะทำให้ประหยัดค่าใช้จ่ายในด้านวัสดุและจำนวนครั้งในการเดินทางมารับการรักษา รวมถึงการลดระยะเวลาการติดเครื่องมือจัดฟันในช่องปาก

ในการวิจัยนี้ ผู้วิจัยได้เลือกใช้แรงสั่นสะเทือนจากแปรงสีฟันไฟฟ้าในการเพิ่มอัตราการเคลื่อนที่ของฟัน เนื่องจากมีรายงานการศึกษาที่แสดงให้เห็นว่า แรงสั่นสะเทือนสามารถเพิ่มอัตราเร็วในการเคลื่อนฟันได้โดยไม่ก่อให้เกิดอันตราย ทั้งนี้ แปรงสีฟันไฟฟ้ายังเป็นอุปกรณ์ที่หาซื้อได้ง่ายและใช้กันโดยทั่วไปในการทำความสะอาดช่องปาก

ท่านได้รับเชิญให้เข้าร่วมการวิจัยนี้เพราะท่านเป็นผู้ที่เข้ามารับบริการที่คลินิกทันตกรรมจัดฟัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ มีอายุระหว่าง 19 – 25 ปี มีสุขภาพร่างกายและสุขภาพช่องปากที่ดี และมีแผนการรักษาที่<u>จะต้องได้รับการรักษาทางทันตกรรมจัดฟันร่วมกับการถอนฟันกรามน้อยบนซี่ที่หนึ่งทั้งด้านซ้ายและขวา</u> เพื่อใช้เป็นที่ว่างในการเคลื่อนฟันเขี้ยวบน ซึ่งในการวิจัยนี้ผู้วิจัยจะทำการพิมพ์ปากเพื่อทำแบบจำลองฟันของท่านเป็นระยะๆ เพื่อทำการเปรียบเทียบระยะทางการเคลื่อนที่ของฟันเขี้ยวบนระหว่างด้านที่ใช้และไม่ใช้การกระตุ้นด้วยแรงสั่นสะเทือนจากแปรงสีฟันไฟฟ้า นอกจากนี้ ผู้วิจัยจะทำการเก็บน้ำเหลืองเหงือกจากบริเวณฟันเขี้ยวบนของท่าน เพื่อนำไปใช้ศึกษาการเปลี่ยนแปลงของระดับไซโตคายน์ซึ่งเป็นชีวโมเลกุลที่มีอยู่ในน้ำเหลืองเหงือก

ทั้งนี้ จะมีผู้เข้าร่วมการวิจัยนี้ประมาณ 15 คน โดย ในระหว่างการรักษาทางทันตกรรมจัดฟันตามปกติ ซึ่งในระยะเวลา 2 ปีโดยประมาณนั้น จะเป็นช่วงเวลาสำหรับการทำวิจัยประมาณ 6 เดือน

หากท่านตัดสินใจเข้าร่วมการวิจัยแล้ว ท่านจะเริ่มต้นเข้าสู่ขั้นตอนการรักษาทางทันตกรรมจัดฟันตามปกติ โดยจะมีการเก็บข้อมูลเบื้องต้นจากการตรวจ ซักประวัติ ถ่ายรูปช่องปากและใบหน้า เอกซ์เรย์ฟันและพิมพ์ปาก สำหรับใช้ประกอบการวางแผนการรักษา

จากนั้น จะมีการนัดหมายเพื่อการรักษาทางทันตกรรมจัดฟัน ซึ่งโดยปกติ จะทำการนัดหมายเดือนละครั้ง ทั้งนี้ในระหว่างทำการรักษา ผู้วิจัยจะขออนุญาตเก็บตัวอย่างน้ำเหลืองเหงือกและพิมพ์ปากในแต่ละช่วงเวลาเพื่อใช้เป็นข้อมูลในการวิจัย

ในวันที่มีการติดเครื่องมือเพื่อให้แรงในการเคลื่อนฟันเขี้ยว ท่านจะได้รับแปรงสีฟันไฟฟ้าเพื่อใช้สำหรับกระตุ้นการเคลื่อนที่ของฟัน

<u>ทั้งนี้ ท่านยังคงต้องรับผิดชอบค่าใช้จ่ายในส่วนของการรักษาทางทันตกรรมจัดฟันตามปกติรวมถึงค่าใช้จ่ายในการเดินทางตลอดระยะเวลาการวิจัยและการรักษา</u>

การใช้แปรงสีฟันไฟฟ้าเพื่อกระตุ้นการเคลื่อนที่ของฟัน

ให้ใช้แปรงสีฟันไฟฟ้าหลังจากที่ท่านแปรงฟันก่อนนอนเสร็จเรียบร้อย กดปุ่มเปิดการทำงานของแปรง นำส่วนของขนแปรง แตะที่ปลายฟันเขี้ยวเบาๆ (เพียงข้างเดียวที่เป็นด้านทดลอง) เป็นเวลา 15 นาทีต่อวัน ติดต่อกันเป็นระยะเวลา 2 เดือน

ผู้ช่วยวิจัยจะเป็นผู้ให้ข้อมูลการใช้แปรง แจกแปรง และแจ้งให้ท่านทราบว่าฟันเขี้ยวด้านไหนที่ใช้ในการวิจัย ซึ่งด้านที่ถูกเลือกเพื่อการวิจัยของท่าน อาจไม่จำเป็นตรงกับผู้เข้าร่วมวิจัยท่านอื่น

หลังจากสิ้นสุดขั้นตอนการวิจัยท่านจะได้รับการรักษาทางทันตกรรมจัดฟันตามปกติ จนเสร็จสิ้นการรักษา

ข้อมูลเกี่ยวกับการเก็บน้ำเหลืองเหงือก

น้ำเหลืองเหงือก คือของเหลวใสๆ ที่มีอยู่ในร่องเหงือกปกติ การเก็บน้ำเหลืองเหงือก ผู้วิจัยจะใช้กระดาษขนาดเล็กดูดซับน้ำเหลืองเหงือก โดยใส่ในร่องเหงือกนาน 1 นาที ซึ่งจะไม่ก่อให้เกิดอาการเจ็บปวดหรือระคายเคืองแต่อย่างใด

ข้อพึงปฏิบัติในระหว่างการวิจัย

1. อย่าใช้แปรงสีฟันไฟฟ้า ทำความสะอาดฟันและช่องปาก (ทั้งแปรงที่ได้รับแจกและแปรงที่ท่านอาจมีอยู่แล้ว) ขอให้ท่านใช้เฉพาะแปรงสีฟันแบบธรรมดาในการแปรงฟันของท่านและใช้แปรงสีฟันไฟฟ้าเพื่อการกระตุ้นฟันเขี้ยวเพียงอย่างเดียว หากแปรงสีฟันไฟฟ้าชำรุด หาย หรือแบตเตอรีหมด ให้โทรแจ้งผู้วิจัย
2. จดบันทึกข้อมูลการใช้แปรง หากมีวันใดที่ท่านลืมใช้แปรงหรือไม่ได้ใช้แปรงเนื่องด้วยเหตุผลบางประการ กรุณาแจ้งให้ผู้วิจัยทราบในการนัดหมายครั้งต่อไป
3. ในระหว่างการวิจัย ควรหลีกเลี่ยงการรับประทานยาในกลุ่ม non steroidal anti-inflammatory drugs (NSAIDs) ตัวอย่างเช่น Ibuprofen, Ponstan, Celeblex เนื่องจากยาดังกล่าวส่งผลกระทบต่ออัตราการเคลื่อนฟัน หากท่านมีความจำเป็นต้องใช้ยาในกลุ่มนี้ในระหว่างการวิจัย กรุณาแจ้งให้ผู้วิจัยทราบ ท่านจะได้รับคำแนะนำให้ใช้ยาพาราเซตามอลหากเกิดความเจ็บปวดอันเนื่องมาจากการรักษาทางทันตกรรมจัดฟัน

ความเสี่ยงที่อาจจะเกิดขึ้นเมื่อเข้าร่วมการวิจัย

การใช้แปรงสีฟันไฟฟ้าตามคำแนะนำ จะไม่ก่อให้เกิดอันตรายใดๆต่อเนื้อเยื่อหรืออวัยวะในช่องปาก แต่การใช้อย่างไม่ระมัดระวัง อาจมีความเสี่ยงที่จะทำให้เกิดการหลุดหรือชำรุดของเครื่องมือจัดฟัน ในระหว่างการใช้แปรงสีฟันไฟฟ้า หากเกิดความเจ็บปวดหรือความผิดปกติใดๆ ให้ท่านหยุดใช้แปรงสีฟันไฟฟ้าและโทรแจ้งให้ผู้วิจัยทราบทันที

หากมีข้อข้องใจที่จะสอบถามเกี่ยวข้องกับการวิจัยหรือเมื่อบาดเจ็บ/เจ็บป่วยหรือเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัย สามารถติดต่อได้ที่ นาย สุมิตร สูอำพัน หรือ รศ.ทพญ.ดร.ชิดชนก ลีธนะกุล ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ เบอร์โทรศัพท์ 074-429875, 081-8363787

หากท่านตัดสินใจไม่เข้าร่วมในโครงการวิจัยนี้ ท่านก็จะได้รับการตรวจเพื่อการวินิจฉัยและรักษาทางทันตกรรมจัดฟันตามวิธีการที่เป็นมาตรฐาน

ท่านมีสิทธิ์ที่จะขอยกเลิกการเข้าร่วมในโครงการวิจัย โดยจะแจ้งให้ทราบล่วงหน้า ซึ่งการยกเลิกนี้จะไม่มีผลต่อการได้รับบริการ หรือการรักษาที่ท่านจะได้รับแต่อย่างใด

หากมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ทราบโดยรวดเร็วไม่ปิดบัง

ข้าพเจ้าได้อ่านรายละเอียดในเอกสารนี้ครบถ้วนแล้วและมีความเข้าใจดี จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ โดยผู้วิจัยได้ให้สำเนาแบบยินยอมที่ลงนามแล้วกับข้าพเจ้าเพื่อเก็บไว้เป็นหลักฐานจำนวน 1 ชุด

.....ผู้ยินยอม

(.....)

…./…./……

แบบบันทึกการใช้แปรงสีฟันไฟฟ้า

...... เดือนที่ 1   ......เดือนที่ 2

ชื่อ - นามสกุล……………………………………………อายุ.........ปี

ด้านที่ใช้แปรง   ...... ซ้าย   ......ขวา

วันที่เริ่มใช้แปรง……………………........   วันที่สิ้นสุดการใช้แปรง……………………

วันที่	เวลาที่ใช้แปรง	รวม (นาที)

Interleukin-1 beta

	Experiment - Pressure (pg/µl)				
Subject No.	baseline	T0	T1	T2+V1	T3+V2
1	0.09	0.04	0.15	0.11	0.14
2	0.02	0.17	0.15	0.12	0.47
3	0.02	0.02	0.12	0.14	0.47
4	0.04	0.02	0.08	0.30	1.02
5	0.03	0.01	0.12	0.19	0.97
6	0.09	0.03	0.13	0.16	0.71
7	0.02	0.06	0.16	0.38	0.43
8	0.03	0.03	0.22	0.13	1.10
9	0.03	0.02	0.13	0.06	0.33
10	0.03	0.06	0.12	0.16	0.26
Mean	0.04	0.05	0.14	0.18	0.59
SD	0.03	0.04	0.03	0.09	0.32

Interleukin-1 beta (continue)

	Experiment - Tension (pg/µl)				
Subject No.	baseline	T0	T1	T2+V1	T3+V2
1	0.02	0.33	0.04	0.24	0.20
2	0.02	0.05	0.03	0.17	0.13
3	0.03	0.01	0.08	0.04	0.14
4	0.02	0.03	0.07	0.02	0.17
5	0.04	0.02	0.02	0.16	0.69
6	0.04	0.08	0.09	0.08	0.25
7	0.03	0.04	0.12	0.22	0.11
8	0.06	0.04	0.17	0.00	0.78
9	0.07	0.09	0.03	0.08	0.45
10	0.00	0.02	0.03	0.04	0.16
Mean	0.03	0.07	0.07	0.11	0.31
SD	0.02	0.09	0.05	0.08	0.23

Interleukin-1 beta (continue)

	Control - Pressure (pg/µl)				
Subject No.	baseline	T0	T1	T2-V1	T3-V2
1	0.01	0.08	0.12	0.08	0.11
2	0.02	0.24	0.25	0.08	0.19
3	0.03	0.02	0.11	0.05	0.06
4	0.06	0.06	0.09	0.21	0.03
5	0.01	0.02	0.06	0.05	0.27
6	0.06	0.03	0.10	0.02	0.00
7	0.01	0.01	0.29	0.05	0.01
8	0.02	0.07	0.17	0.03	0.21
9	0.03	0.02	0.08	0.11	0.01
10	0.09	0.04	0.34	0.02	0.03
Mean	0.03	0.06	0.16	0.07	0.09
SD	0.03	0.07	0.09	0.05	0.09

Interleukin-1 beta (continue)

	Control - Tension (pg/µl)				
Subject No.	baseline	T0	T1	T2-V1	T3-V2
1	0.01	0.04	0.09	0.04	0.00
2	0.02	0.08	0.08	0.10	0.25
3	0.03	0.01	0.04	0.03	0.03
4	0.06	0.05	0.04	0.11	0.25
5	0.08	0.01	0.03	0.08	0.10
6	0.02	0.02	0.02	0.05	0.01
7	0.02	0.05	0.01	0.08	0.00
8	0.02	0.06	0.05	0.25	0.25
9	0.03	0.02	0.06	0.05	0.15
10	0.02	0.14	0.07	0.15	0.01
Mean	0.03	0.05	0.05	0.09	0.11
SD	0.02	0.04	0.02	0.06	0.11

Rate of tooth movement

Subject No.	T2+V1	T2-V1	T3+V2	T3-V2
1	0.91	0.96	0.53	0.64
2	1.13	0.77	0.89	0.39
3	1.33	0.73	0.86	0.51
4	1.06	0.62	1.25	0.54
5	1.33	0.83	1.02	0.65
6	0.84	0.61	0.84	0.57
7	1.2	0.91	1.11	0.87
8	1.18	1.19	0.8	0.89
9	1.82	1.02	0.93	0.65
10	1.25	0.85	1.14	0.47
Mean	1.21	0.85	0.94	0.62
SD	0.26	0.17	0.19	0.15

VITAE

Name Mr. Sumit Suamphan

Student ID 5010820017

Educational Attainment

Degree	Name of Institution	Year of Graduation
Doctor of Dental Surgery	Chulalongkorn University	2547

List of Publication and Proceeding

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