A Comparative Study of Different Vibratory Stimuli Frequencies during Orthodontic Tooth Movement on the Secretion of Receptor Activator of Nuclear Kappa-B Ligand (RANKL)/Osteoprotegerin (OPG) Ratio

Natchanon Siriphan

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Oral Health Sciences

Prince of Songkla University

2019

Thesis Title A comparative study of different vibratory stimuli frequencies during orthodontic tooth movement on the secretion of Receptor Activator of Nuclear Kappa-B Ligand (RANKL)/Osteoprotegerin (OPG) ratio

Author Mr.Natchanon Siriphan

Major Program Oral health science

Major Advisor

.....

(Assoc. Prof. Dr. Udom Thongudomporn)

Examining Committee:

.....Chairperson

(Prof. Dr. Smorntree Viteporn)

.....Committee

(Prof. Dr. Steven J. Lindauer)

.....Committee

(Assoc. Prof. Dr. Udom Thongudomporn)

.....Committee

(Asst. Prof. Dr. Bancha Samruajbenjakun)

The Graduate School, Prince of Songkla University, has approved this thesis as Partial fulfillment of the requirements for the Doctor of Philosophy Degree in Oral health science

…….....

(Prof. Dr. Damrongsak Faroongsarng)

Dean of Graduate School

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

.....Signature

(Assoc. Prof. Dr. Udom Thongudomporn)

Major Advisor

.....Signature

(Mr. Natchanon Siriphan)

Candidate

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

…….....Signature

(Mr. Natchanon Siriphan)

Candidate

ชื่อวิทยานิพนธ์ การศึกษาเปรียบเทียบการกระตุ้นการเคลื่อนฟันทางทันตกรรมจัดฟันด้วยการสั่นที่ความถี่ต่างๆ ต่ออัตราส่วนการหลั่งของ แรงค์แอลต่อโอพีจี

ผู้เขียน นายณัฐชนน ศิริพันธ์

สาขาวิชา วิทยาศาสตร์สุขภาพช่องปาก

ปีการศึกษา 2561

บทคัดย่อ

การที่จะเร่งการเคลื่อนฟันในการรักษาทางทันตกรรมจัดฟันมีอยู่ด้วยกันหลายวิธี การสั่นเป็นวิธีการหนึ่งที่จะช่วยกระตุ้นในการเคลื่อนฟันซึ่งหลายๆ การศึกษาในปัจจุบันยังให้ผลของการกระตุ้นการเคลื่อนฟันไม่ไปในทิศทางเดียวกัน ซึ่งขึ้นอยู่กับหลายๆ ปัจจัยและปัจจัยหนึ่งที่มีผล คือ ความถี่ นอกจากนี้ยังมีการศึกษาที่น้อยที่ศึกษาผลของการสั่นต่อการเคลื่อนฟันร่วมกับการหลั่งไซโตไคล์ (cytokine) ในทางคลินิกร่วมด้วย **วัตถุประสงค์** เพื่อศึกษาผลของการกระตุ้นด้วยการสั่นที่ความถี่ 30 เฮิร์ต (Hertz, Hz) และ 60 เฮิร์ต ต่ออัตราการเคลื่อนฟันเขี้ยวบน และการหลั่งของ แรงค์แอล (RANKL) และโอพีจี (OPG) **วิธีการวิจัย** กลุ่มตัวอย่าง จำนวน 60 คน อายุเฉลี่ย 21.5 ± 2.0 ปี มีแผนการรักษาทางทันตกรรมจัดฟันที่ต้องถอนฟันกรามน้อยบนซี่แรกและเคลื่อนฟันเขี้ยวบนโดยใช้แรง 60 กรัม บนเครื่องมือจัดฟันแบบติดแน่นขนาด 0.022 นิ้ว ด้วยลวดสแตนเลสสตีลขนาด 0.016 x 0.022 นิ้ว หลังทำการเรียงฟันจนได้ระดับที่ดีแล้ว และกลุ่มตัวอย่างทั้งหมดถูกสุ่มเข้ากลุ่ม 3 กลุ่ม กลุ่มละ 20 คน คือ 1. ทำการเคลื่อนฟันเขี้ยวบนร่วมกับการสั่นที่ 30 เฮิร์ต 2. ทำการเคลื่อนฟันเขี้ยวบนร่วมกับการสั่นที่ 60 เฮิร์ต 3. ทำการเคลื่อนฟันเขี้ยวบนเพียงอย่างเดียว ในช่วง 7 วันแรกของการเคลื่อนฟันเขี้ยวบนจะถูกนำมาวิเคราะห์ด้วยวิธีการเปอโปโตคอล (per-protocol analysis) และขั้นตอนการสั่นผู้ทำการทดลองจะเป็นคนแตะเครื่องสั่นที่ฟันเขี้ยวเป็นเวลา 20 นาทีต่อวันที่เวลาเดียวกัน และทำการเก็บน้ำเหลืองเหงือกเพื่อนำมาวิเคราะห์ปริมาณแรงค์แอลและโอพีจีในช่วงเวลา ก่อนทำการเคลื่อนฟันเขี้ยว (T1), หลังเคลื่อนฟันเขี้ยวไปแล้ว 24 ชั่วโมง (T2), หลังเคลื่อนฟันเขี้ยวไปแล้ว 48 ชั่วโมง (T3), หลังเคลื่อนฟันเขี้ยวไปแล้ว 7 วัน (T4) และหลังจากวันที่ 7 เป็นต้นไป กลุ่มตัวอย่างที่ได้รับเครื่องมือสั่นต้องใช้เครื่องมือสั่นที่บ้านด้วยตัวเองเป็นเวลา 20 นาทีต่อวันตามคำแนะนำ อัตราเร็วของการเคลื่อนฟันเขี้ยวบนทำการคำนวณจากเครื่องสแกนดิจิตัลโมเดลที่เวลา T1 กับ 3 เดือนหลังจากเคลื่อนฟันเขี้ยว (T5) และข้อมูลถูกนำมาวิเคราะห์แบบอินเท็นชั่นทูทรีท (intention-to-treat) ตัวแปรทั้งหมดมีการกระจายของข้อมูลไม่ปกติ การทดสอบของครัสคาลและวัลลิส ใช้ในการวิเคราะห์ความเปลี่ยนแปลงระหว่าง 3 กลุ่ม และการเปลี่ยนแปลงระหว่างช่วงเวลาทั้ง 3 กลุ่มโดยมีนัยสำคัญที่ระดับ 0.05 **ผลการศึกษา** อัตราเร็วของการเคลื่อนฟันเขี้ยวบนไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่ม ($P>0.05$) แรงค์แอล, โอพีจี และสัดส่วนแรงค์แอลต่อโอพีจี ไม่พบความแตกต่างอย่าง

มีนัยสำคัญทางสถิติระหว่างกลุ่มที่แต่ละช่วงเวลาหรือภายในกลุ่มที่ระหว่างช่วงเวลา (P>0.05) ยกเว้นแรงค์แอลบนด้านที่ออกแรงกด (compression side) พบมีความแตกต่างอย่างมีนัยสำคัญทางสถิติที่ T2, T3 และ T4 ที่มากกว่า T1 ในกลุ่มควบคุม (P<0.001) **สรุปผลการศึกษา** ผลของการสั่นที่ความถี่ 30 เฮิร์ตและ 60 เฮิร์ตไม่พบมีผลต่อการกระตุ้นการเคลื่อนฟันเขี้ยวบน, การหลั่งของแรงค์แอล, โอพีจี หรือสัดส่วนแรงค์แอลต่อโอพีจีในทางคลินิก

Thesis Title A Comparative Study of Different Vibratory Stimuli Frequencies during Orthodontic Tooth Movement on the Secretion of Receptor Activator of Nuclear Kappa-B Ligand (RANKL)/Osteoprotegerin (OPG) Ratio

Author Mr. Natchanon Siriphan

Major Program Oral Health Sciences

Academic Year 2018

ABSTRACT

The accelerated orthodontic tooth movement had several methods. The vibration is one method that is used to accelerate tooth movement. However, previous studies showed controversy results because of several factors. The one factor had also affected which was frequency. In addition, there were a few studies that investigated the effect of vibration to tooth movement and cytokine secretion in clinical setting. **Objectives:** To investigate the effect of vibratory stimuli frequencies at 30 Hz and 60 Hz on the rate of maxillary canine distalization and secretion of RANKL and OPG. **Methods:** 60 subjects (age 21.5 ± 2.0 years) who received orthodontic treatment plan which was upper first premolar extraction and canine distalization by 60 grams force on 0.022" pre-adjusted edgewise appliance with 0.016" x 0.022" SS archwire after completed leveling and aligning. All participants were randomly selected to 3 groups 1. Canine distalization + vibration 30 Hz. 2. Canine distalization + vibration 60 Hz 3. Canine distalization only. For seven days after started, canine distalization was analysis with per-protocol analysis. Examiner applied vibration for 20 minutes/day at the same time. Gingival crevicular fluid was collected to analysis RANKL and OPG on before distalization (T1) and 24 h (T2), 48 h (T3), and 7 days (T4) after canine distalization. After seven days, participants applied vibration with themselves at home following the recommendation. The rate of maxillary canine distalization was calculated with digital models T1 to 3 months after canine distalization (T5). The data was analyzed with (intention-to-treat) . All parameters were non – normal distribution, the changes parameter and different time-point among 3 groups were examine by Kruskal-Wallis test at significant level 0.05. **Results:** The maxillary canine distalization rate did not statistically significant different between group ($p>0.05$). RANKL, OPG and RANKL/OPG ratio were not statistically significant different between groups and intragroup at

each time-points ($p>0.05$) Nevertheless, RANKL on the compression side was statistically significantly higher at T2, T3 and T4 than T1 in the control group ($p<0.001$). **Conclusions:** The effect of vibratory stimuli frequencies at 30 Hz and 60 Hz combined with light orthodontic force (60 grams) for 3 months did not significantly accelerate the rate of canine distalization when compared to 60 grams of orthodontic force alone. In addition, the RANKL and OPG or the RANKL/OPG ratio expression did not statistically significant for 7 days vibratory stimuli application.

ACKNOWLEDGEMENT

As the author, I am honoured to thank Faculty of Dentistry, Prince of Songkla University for this remarkable opportunity.

I would like to express my gratitude and appreciation to *Assoc. Prof. Dr. Udom Thongudomporn*, my advisors for your guidance, supervision, invaluable advice and encouragement. I also would like to thank *Assoc. Prof. Dr. Chidchanok Leethanakul* for her knowledges, suggestions and all assistance to make completely this research. I would like to special thanks to *Professor Smorntree Viteporn and Professor Steven J Lindauer* who gave worthy advice and comments and encouragement. In addition, I would like to give thanks for Asst Prof. Dr. Chatchai Putson who suggested to vibrator testing and development. Moreover, I would like to give a lot of thank to the staffs at the research center and orthodontic clinic, Faculty of Dentistry, Prince of Songkla University for help and support. Thank you, all participants, this thesis could not be finished without their cooperation.

I also would like to thank my parents, brother and dear for all their encouragement, patience and support.

Finally, I would like to acknowledge the Graduate School, Prince of Songkla University for the provision of all essentials for achievement of my Ph.D.

Natchanon Siriphan

CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF FIGURES (CONTINUED)

Figure Page

LIST OF ABBREVIATION AND SYMBOLS

WBV	Whole-body vibration
GCF	Gingival crevicular fluid
RANK	Receptor Activator of Nuclear Factor-Kappa B
sRANKL	Soluble Receptor Activator of Nuclear Factor-Kappa B Ligand
RANKL	Receptor Activator of Nuclear Factor-Kappa B Ligand
hRANKL	Human Receptor Activator of Nuclear Factor-Kappa B Ligand
OPG	Osteoprotegerin
PDL	Periodontal ligament
g/cm^2	Gram/ square centimeter
µl	Microliter
mm	Millimeter
ICC	Intraclass correlation coefficient
Pg/ml	Picogram per milliliter
ELISA	Enzyme-linked immunosorbent assay
IL-1β	Interleukin-1Beta
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-11	Interleukin-11
TNF-α	Tumour Necrosis Factor
VEGF	Vascular endothelial growth factor
GFs	Growth factors
FGF-2	Fibroblast growth factor 2
IGF-1	Insulin-like growth factor 1
EGF	Epidermal growth factor
ICAM-1	Intercellular adhesion molecule 1
VCAM-1	Vascular cell adhesion molecule 1
PGE_2	Prostaglandin E2

LIST OF ABBREVIATION AND SYMBOLS (CONTINUED)

CCL2	Chemokine (C-C motif) ligand 2
CCL3	Chemokine (C-C motif) ligand 3
CCL5	Chemokine (C-C motif) ligand 5
CCL7	Chemokine (C-C motif) ligand 7
CCL9	Chemokine (C-C motif) ligand 9
CXCL10	Chemokine (C-X-C motif) ligand 10
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCL13	Chemokine (C-X-C motif) ligand 13
M-CSF	Macrophage colony-stimulating factor
c-fms	Colony stimulating factor-1-receptor
TRAP	Tartrate-resistant acid phosphatase
COX-2	Cyclooxygenase 2
TGF-β	Transforming growth factor beta
BMP	Bone morphogenic protein
BMP-2	Bone morphogenic protein 2
RAP	Regional acceleratory phenomenon
AAO	Accelerated osteogenic orthodontics
LLLT	Low level laser therapy
V	Volt
ω	Omega
m/s^2	meter per square second
t	Time
h	Hour
n	Number
f	Frequency
Hz	hertz
M	Meter
m/s	Meter per second
PMP	Post-menopause

LIST OF ABBREVIATION AND SYMBOLS (CONTINUED)

BFR	Bone formation rate
OVX	Ovariectomize
BMD	Bone mass density
mg/ml	Milligram per milliliter
cm	Centimeter
MS	Mineralized surface
BS	Bone surface
BV	Bone volume
TV	Total bone volume
CD1, C57BL, BALB/cByJ	Rat species
Yr	Year
Wk	Week
D	Day
OSX	Osterix
RUNX2	Runt-related transcription 2
mV	Millivolt
CU-NiTi	Copper Nickle Titanium
KFF	Kinetic frictional force
SSP	Stick-slip Phenomenon
SFF	Static frictional force
PEMF	Pulped-electromagnetic fields
μm	Micrometer
cN	Centinewton
NiTi	Nickle titanium
SN-GoMe	Angle between sella-nasion plane and mandibular plane
FMA	Angle between Frankfort horizontal plane and mandibular plane
Occl-SN	Angle between sella-nasion plane and occlusal plane
SNA	Angle between sella-nasion-point A

LIST OF ABBREVIATION AND SYMBOLS (CONTINUED)

SNB	Angle between sella-nasion-point B
ANB	Angle between point A-nasion-point B
ANS-PNS	Palatal plane
ANS	Anterior nasal spine
PNS	Posterior nasal spine
DNA	Deoxyribonucleic acid
mRNA	Messenger ribonucleic acid
PBS	Phosphate buffer saline
TRAFs	Tumour necrosis factor receptor associated factors
NF-kappaB	Nuclear Factor-Kappa B
AAP	American academy periodontology
PP	Per-protocol analysis
ITT	Intension-to-treat analysis
IQR	Interquartile range
M	Male
F	Female
P	P-value
SD	Standard error
%CV	Percent coefficient of variance
ANOVA	One-way analysis of variance
ME	Method error
ICC	Intraclass correlation coefficient
T1	Immediately before canine distalization
T2	24 hours canine distalization
T3	48 hours canine distalization
T4	7 days canine distalization
T5	3 months canine distalization
g	Gram
N	Newton

LIST OF ABBREVIATION AND SYMBOLS (CONTINUED)

%	Percentage
°C	Celsius degree
s	Second
min	Minute
º	Degree
“	Inch
3D	Three dimension
h	Hour

CHAPTER 1

INTRODUCTION

Background to the problems

Orthodontic treatment is a method to solve malocclusion by generating tooth movement. The process occurs through the mechanical stimuli passing to each tooth and surrounding periodontium of the tooth in the biological level.[1] The signals that control orthodontic tooth movement are conducted from periodontal ligament cells and transferred to the target cells (bone cells), resulting in bone remodeling.[2] When the periodontal ligament is compressed and stretched, bone resoption and formation occurred, respectively, so the tooth moves with remodeled bone to the equilibrium position.[3]

There are 3 main theories that explain the mechanism of orthodontic tooth movement. 1) Piezoelectric theory proposes that electrical charge is generated from the applied forces (stress and strain), which stimulate the rearrangement of cells and increase their convexity.[4] The animal experiment had confirmed that the electric potential was found in the mandible of dogs after the force was applied, the bending of alveolar bone occurred, the electronegative charge and osteoblast cells were found on the concave side, whereas the electropositive charge and osteoclast cells were found on another side.[5] 2) Bone bending theory describes that the adaptation of surrounding tooth structures occurs after they receive the force. As the bone is more flexible than the tooth and other solid structures around the tooth, the bone that bend leads to stretching of periodontal ligament on the concave side resulting in bone formation. The convex side shows the compression of periodontal ligament and the bone resorption takes place.[93, 94] 3) Pressure tension theory explains that the compressed periodontal ligament induces osteoclastogenesis on the compression side, where the osteoclast cells fill in the target areas to stimulate frontal bone resorption. On the tension side, the periodontal ligament is stretched and signal for osteogenesis then gathers osteoblast cells for bone formation.[1, 6] All of these processes involve alteration of blood circulation and mediators from stimulated cells.

The orthodontic treatment usually occupy 2-3 years treatment period,[7, 8] which is considered to be long enough to cause side effects from the treatment such as dental

caries,[9, 10] root resorption and periodontal disease.[11, 12] In a circumstance that the reduced treatment time, decreased side effects, less bone traumatic, promoted patient's satisfaction. The method to accelerate tooth movement has been introduced which include the modification in biological response and bone remodeling. The successful of the results can provide great benefit to both patients and orthodontists.

Many studies had proven the effective tooth movement by increasing rate of the bone remodeling via the increase in cell activity, which correlated to the release of inflammatory cytokine such as Corticotomy,[13, 14] Low level laser application,[15-17] Electrical current,[18] Distraction osteogenesis,[19-22] and Pulp electromagnetic field.[23, 24] However, these techniques are not usually occupied in conventional orthodontic treatment procedure because the side effects are still unpredictable. The amount of published results is also limited due to ethical issues in human study. For less invasive intervention such as, the physical and mechanical device-assisted treatments have been generally used for the accelerated tooth movement instead of additional procedure.

The vibration is the method of choice for this study and non-invasive method to increase rate of tooth movement. The use of vibration has been reported since 1980 for medical purpose; the whole-body vibration (WBV) has been experimented on animal and human bones. Its mechanism was based on the principle of Wolff's law which explains the ability of trabecular bone that can adapt to exogenous stimuli,[25] meaning that a proper amount of mechanical strain is able to induce the change in bone morphology. Osteoporosis can be treated by low magnitude and high frequency vibration. The outcome of treatment promoted osteogenesis process and decreased osteoclastogenesis process in craniofacial structure.[26-28, 29, 30, 31] In addition, vibration alone on the periodontal ligament (PDL) cells demonstrated that vibration could promote anabolic effect.[32-34] However, in dental study, Shapiro et al.,[35] found that when exogenous stimuli were continuously applied to the tooth, piezoelectricity is not generated. Nicolella DP et al[27] and Uzer G et al[28] found that vibrational appliance can be used instead to induce electric charge or osteocyte filtration in the lacunar-canalicular network for rapid bone remodeling response,[36, 37] proliferation of blood vessels,[38] increasing cell activity,[39] increasing inflammatory cytokine[40] which the same results as orthodontic tooth movement. In addition, in vitro studies, when applied vibration combined with compressive force, vibration could increase the inflammatory cytokine to induce osteoclstogenesis in the PDL cells.[41, 42] Animal studies found that combination between vibration and compressive force has been

revealed the controversy. Some studies significantly increased rate of tooth movement[43-45] and others did not find significant difference to affect tooth movement.[46, 47] In clinical studies, the results showed the inconsistency of ability of vibration combined with orthodontic force to reduce the irregularity and accelerate tooth movement. In assessing the alignment of anterior teeth did not significantly reduce the crowding when orthodontic force alone compared to vibration combined with orthodontic force.[48-51] While the distance of tooth movement was reported in controversial results.[52-54] That might be due to a variation in protocols, orthodontic mechanics and outcome measurement.

Although, the disparities of results of effect vibratory stimuli on the rate of tooth movement, it had some studies, examined the effect of vibratory stimuli on the rate of canine distalization.[53-55] There was one study that studied the effect of vibration on the secretion of interleukin-1 beta (IL-1β) and rate of tooth movement.[53] Even though IL-1β could stimulate osteoclastic activity but the receptor of osteoclast did not specific to the osteoclastic activity. Therefore, to specific osteoclastogenesis response to vibration, the levels of receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) are directly measured and may demonstrate important information. RANKL stimulates the function, activation, survival, and differentiation of osteoclast precursors to mature osteoclasts to promote bone resorptive processes, while OPG acts as a decoy receptor to regulate the termination of osteoclastic activity and promotes osteogenic activity.[56, 57]

Consequently, the previous study also indicated that the different frequencies of vibration shows variation in tooth movement and is still controversial. Moreover, there is no study that describes the effect of vibration combines with orthodontic force on the expression of RANKL/ OPG ratio in human. Therefore, the purpose of this study is to define the effect of 30 Hz and 60 Hz vibration combined with light orthodontic force on the rate of maxillary canine distalization and secretion of RANKL and OPG.

Review literature

1. Theory of tooth movement

There are 3 main theories that clarify the mechanism of orthodontic tooth movement.

1.1 Pressure tension theory explained that force pass to the tooth and produces the tooth movement causing the alteration in periodontal ligament. This process

leads to bone remodeling that periodontal ligament at compression side, disorganized and decreased cell replication because of vascular constriction and induce osteoclastogenesis which moves the osteoclast cells into target area for function and produces frontal bone resorption.[6] The other side is tension side where the periodontal ligament is stretched. Cell replication and periodontal ligament production are increased. The signals are transmitted and induced osteogenesis process. Finally, the osteoblast cells are elevated to generate bone formation.[6] All of these processes involve alteration of blood circulation and mediators from stimulated cells.

Besides, magnitude of orthodontic force has a correlation to tissue response. When the force is applied to the tooth, if it is more than capillary blood pressure (20-25 g/cm^2 of root surface), tissue will be necrosis and leads to undermining resorption or hyalinization.[58]

1.2 Bone-bending theory was proposed in 1969 by Baumrind,[6] who mentioned that part of periodontium in pressure-tension theory can be developed to the solid-bone and tooth. The deflection of alveolar bone and changes in periodontal ligament can be stimulated by orthodontic force. Later, in 1888 Farrar[59] proposed that bending of alveolar bone is an important role in orthodontic tooth movement. This concept was proven by experiment of Baumrind[6] and Grimm.[60] The opposite direction to tooth movement when applied orthodontic force was represented the deflection on the socket wall.

In the concept of bone-bending theory, from Pascal's law, force spreads equally to every area that force is applied. So, forces are delivered to the tooth and transmitted to periodontal ligament around the tooth. And force cannot change configuration of periodontal ligament due to containing with liquid and ground substance that join tooth, bone and periodontal ligament together. It is called hydrostatic system. When periodontal ligament is compressed, fluid system will move to other side. It means that the pressure in the periodontal ligament equals to all regions, however the bone is bent. Consequently, forces can bend the periodontium such as bone, tooth and discrete solid fractures of periodontal ligament to the solid parts. And each of three types structure is deformed. The amount of deformation is depended on the elastic properties of each structure. It assures that bone has bent far more than periodontal ligament because periodontal ligament joins tooth and bone to one unit that becomes bone can bend more than periodontal ligament. The biological response involves turnover and renewal of bone that have occurred in deformed bone position. The alteration of shaped alveolar bone creates a concave and convex surface that are identified in the long

bone. The concave pattern is the stretching of periodontal ligament and molecules are compressed i.e. the apposition of alveolar bone takes place whereas the convex pattern has a compressed PDL means that resorption of alveolar bone has occurred.[4, 5, 61, 62]

1.3 Piezoelectric theory was proposed by Bassett and Becker[4] in 1962 that the electrical charge is risen by applied forces (stress or strain) in stress tissue that response of cell rearrangement and increasing convexity. Additionally, the experiment in dog's mandible by Zengo et al.[5] found electric potential after force was applied to the tooth from bending of alveolar bone. The concave side presents electronegative charge and fulfills with osteoblasts. However, the other side found electropositive charge and elevated the osteoclasts. Davidovitch has proposed that there is an elevation of cellular response in periodontal ligament and alveolar bone from electrical current combination with orthodontic force.[63] These findings indicate that bending of alveolar bone results from bioelectric activity occurred by applying orthodontic force and may have a role as essential cellular first messengers.

Although, all of phenomenons showed physical and biological activity that has been known as theories or unknown other phenomena, they initiated from mechanical forces that activate concurrently cells, periodontal ligament and bone to release mediators and create remodeling activity, which enables tooth movement.

2. Optimal orthodontic force

The optimal force for the highest efficiency for orthodontic tooth movement is that the tooth can be moved without creating patient's discomfort and tissue damage.[64] The force has the light and heavy forces; the optimal force is not possible to be measured precisely with available instrument when force was applied to the root. Burstone[65] stated that periodontal ligament is not equally supported orthodontic force in each root surface. And favorable biological response of periodontal ligament produces from the light force because it is appropriate to generate frontal bone resorption. However, heavy force can cause necrosis of periodontal ligament and undermining bone resorption,[1, 66] which can lead to root resorption.

The concept of optimal force, according to Schwarz[58] stated that the force leads to a change of tissue pressure which determined by capillary vessels and blood pressure. Any forces that are lower than the optimal force would generate no reaction and no tooth movement.[67] Meanwhile the forces that are higher than optimal force would cause tissue necrosis and decrease rate of tooth movement.[67]

Consequently, the current concept of optimal orthodontic force is mechanical stimulus that activates the process of cellular response to re-establish balance by remodeling of periodontium, maximize the rate of tooth movement, provide adequate patient comfort and minimize damage to periodontal supporting tissue.[3, 68] This concept may be possible by defining a certain force magnitude and characteristic of applied force to use. However, the optimal force might be different in each patient and each tooth. Therefore, optimal orthodontic force can be identified according to the relationship both force magnitude and rate of tooth movement which are considered to be a practical guide in order to produce effective tooth movement and no overload to periodontal tissue.

From the literature reviews about canine retraction[68] found that the initial force can move canine about 18-1500 grams. The heavy force can damage to periodontal supporting tissue, undesirable tooth movement and create pain. The light force can produce effective of biology, safe to periodontal supporting tissue and less painful and better comfort however it is not confirmed that the tooth moves if the light force cannot overcome the friction between wire and bracket. So, the force is appropriate to move canine and less side effect when combine with the vibration about 60 grams from the study of Leethanakul et al. 2015[53]and Insee K et al 2014.[69] Because 60 grams of force is used with the stiff wire can move the tooth. In addition, 60 grams of force does not to damage to periodontal supporting tissue if the vibration force is combined to orthodontic force.

3. Process of orthodontic tooth movement and cellular response

The surrounding tooth structures such as periodontal ligament, dental pulp, alveolar bone and gingiva response to orthodontic force. Applying force on the tooth triggers the periodontal ligament cells to generate the signal transferred to surrounding bone for the remodeling process. The rate of tooth movement may be changed depending on the amount, frequency and duration of force combined with the biological response of the periodontal ligament. Since the application of force effect the expression of periodontal ligament cells, different molecules such as cytokine and growth hormone are synthesized and released to evoke cellular response around the teeth to provide proper tissue adaptation.[40,63]

The inflammatory mediator's secretion of orthodontic tooth movement

When the tooth is received orthodontic force, the periodontal ligaments response immediately. The compression side shows tissue and cell damage with the change of the capillary by reducing blood flow leading to ischemia and hypoxia.[70] Then, the body reacts by increasing vasodilatation of blood vessels and migration of leukocytes. This process is activated by interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF) expression in periodontal ligament fibroblasts.[71] In the meanwhile, physical strain stimulates the product of growth factors (GFs), cytokines and chemokines through the process of mechanotransduction.[70] IL-1β and TNF-α stimulate the endothelial cells and the adhesion molecule expression (ICAM-1 and VCAM-1) that trigger expression of chemokine, adhesion and migration of leukocyte.[72] In addition, periodontal ligament, peripheral nerves release neurotransmitters such as Calcitonin gene-related peptide and substance P.[70] These neuropeptides combined with VEGF and Prostaglandin E_2 (PGE_2), lead to the increase of vascular permeability, and blood flow and leukocyte diapedesis.[73] These leukocytes interact with paradental cells and increase the chemokines, cytokines, and GFs for bone resorption. After that, the acute inflammatory process is replaced with chronic inflammation that permits leukocytes and osteoclast precursors migrate to the target for remodeling process.[70]

Bone resorption

The mechanical stimulus is loaded to the fibroblast, osteoblast and other periodontal ligament cells in the pressure side. These cells release the signaling molecules such as PGE_2, IL-1, IL-6, TNF-α and IL-11.[72, 74, 75] Chemokine (CCL3, CCL2, and CCL5) are produced by osteoblast after the IL-1 and TNF-α are activated.[72, 75-77] Then, CXCL12 and RANKL and TNF-α are combined with the chemokines (CCL3, CCL2 and CCL5) and induce enrollment of osteoclast precursors to the resorptive site and then differentiate into the mature osteoclasts by communication of osteoblasts and osteoclasts.[78-80] The differentiation of osteoclasts is regulated from the main cytokines which are PGE_2, IL-1, IL-6, IL-8 and TNF-α that stimulate osteoblast or stromal cells to produce the RANKL and macrophage colony-stimulating factor (M-CSF).[81-84] The process is achieved by M-CSF and RANKL that bind to their specific receptors, colony stimulating factor 1 receptor (c-Fms) and receptor activator of nuclear factor kappa B (RANK) which express on the surface of osteoclast precursors but osteoclastogenesis will be down-regulated by osteoprotegerin (OPG). It is a RANKL decoy

receptor that produced by osteoblast and periodontal ligament. The process completes when RANKL binds with RANK receptor for inhibiting RANK/RANKL interaction.[82]

The force also induces hypoxia that expresses the factor1-α. It stimulates the expression of RANKL via periodontal ligament and enhances osteoclastogenesis.[85] In addition, orthodontic force can cause microdamage in alveolar bone and make damaging cells by oxidative stress or disrupted blood flow in lacunar-canalicular system. So, osteocytes are activated and secrete the RANKL and M-CSF.[86] This damaged tissue that combined with TNF-α and IL-1 can stimulate apoptosis of osteocytes, which induces bone resorption near the area of microdamage. And it is found that up-regulation of RANKL, VEGF, and M-CSF trigger recruitment of osteoclast precursors and differentiation.[86] Furthermore, VEGF initiates the angiogenesis in the resorption area by recruitment of osteoclast precursors.[86]

The other cytokines (IL-β, TNF-α, IL-6, IL-11), growth factors (FGF-2, EGF) and chemokines (CCL2, CCL3, CCL5, CCL7, CCL9, IL-8) can also stimulate the osteoclast differentiation, survival and activity.[78, 87, 88]

Bone formation

When the PDL is received the orthodontic force. Bone formation process can occur, as osteocytes in the alveolar bone near the periodontal ligament are sensitive to the tensile force. They communicate with neighboring osteocytes, alveolar bone surface-lining cells, and bone marrow cavity cells. Osteoblasts response to these signal and initiate bone formations which direct contact to the osteocyte.[89] Besides, cell replication can be arisen by stretched periodontal ligament fiber.[2] Stem cells (pericyte) drift from blood vessel to active site and differentiate to preosteoblast cell.[2] Then, CCL3, CCL5, CXCL10, CXCL12, and CXCL13 markers induce the process of osteoblast precursor recruitment, proliferation, differentiation and survival.[77, 89, 90] Transforming growth factor-β (TGF-β) and Insulin-like growth factor-1 (IGF-1) are secreted from osteoblasts and osteocytes that induce proliferation and differentiation of osteoblast precursors to mature osteoblasts and mineralization of new bone.[91-93] Furthermore, bone morphogenic protein (BMP), epidermal growth factor and IL-11 can control differentiation and function of osteoblasts.[94-96] The osteoblasts and periodontal ligament fibroblasts create the VEGF that initiates angiogenesis process result in new bone formation.[73, 97] Also osteoblasts produce IL-10 and OPG involve in osteogenesis which inhibits osteoclastogenesis.[82, 98] Moreover, to maintain periodontal ligament apparatus, TGF-β and IGF-1 can stimulate function of osteoblasts and PDL cells proliferation and differentiation and collagen synthesis.[99-101]

RANKL/OPG ratio during orthodontic tooth movement.

Previous studies showed that applying force group to move the teeth had an increased level of RANKL at 24 hours,[102, 103] 48 hours[104] and 42 hours when compared to the non-applying force group.[105] In addition, RANKL level in adults was less than in juveniles[102, 103, 106] and the ratio RANKL/OPG increase at 6 weeks in adolescent[107] after rectangular was placed because higher alveolar bone turnover rate of periodontium in adolescents compared with adults. The studies of OPG level found that the application of mechanical stress group decreased the level of OPG at 1 hours,[108] 24 hours,[103, 108, 109] 7 days,[108] 1 months[108] and 3 months[108] than the non-application of mechanical stress group and level of OPG and RANKL/OPG ratio in adults were lower than juveniles[103] due to decreased function of periodontal ligament cells proliferation, the amount of collagen, organic matrix and alkaline phosphatase.[110, 111] The site specification determined the levels of RANKL and OPG.[112] However, the level of cytokines was down-regulation after reaching the peak values, mostly 24 hours (continuous force) if the activation (interrupted force) is repeated. The up-regulation of cytokine is increased.[106] In addition, Kim et al.[113] studied on the compressive force encouraged the increased M-CSF, RANKL, TNF-α and decreased OPG in periodontal ligament cell via the integrin-FAK pathway, found that Integrin-FAK pathway regulates the expression of M-CSF, RANKL, TNF-α and OPG by increased M-CSF, RANKL, TNF-α and decreased OPG when compressive force was applied. So, the RANK-RANKL and OPG pathway can activate or inhibit orthodontic tooth movement via the RANKL/OPG ratio expression. And it has the effect to the rate of tooth movement.

Phase of tooth movement

In the past, tooth movement could divide into 3 phases.[2] The initial phase is a tooth movement after the force is immediately applied to the tooth. The tooth will move in the periodontal ligament space. The lag phase is immediately occurred after the initial phase and created slow speed of tooth movement. Due to the periodontal ligament hyalinization in the compression side is produced. The tooth cannot move until the hyalinization area will be removed. The third phase occurs after the lag phase. The tooth will suddenly or gradually move and increase the rate of orthodontic tooth movement. There are 2 studies[114, 115] that present a new model of tooth movement. The phase of tooth movement from 2 studies can divide 4 phases of tooth movement the first phase has occur in 24 hours to 2 days that it seems as the first phase is mentioned above. The tooth will move into the socket. The second phase

takes time 20-30 days and the tooth cannot move. After that, the third and fourth phase find that the tooth is accelerated tooth movement when removed hyalinization. It exhibits around 40 days after applied the initial force. The second to fourth phase is same as the lag phase and the post-lag phase are mentioned above.

To investigate and measure the expression of RANKL/OPG ratio follows the previous study and phase of tooth movement. The day on 24 hours, 48 hours 7 days and 30 days is possible to find expression of RANKL/OPG ratio by regardless of frontal bone resorption or undermining resorption.

4. Methods of orthodontic tooth movement acceleration

4.1 Surgical approach

4.1.1 Corticotomy

Corticotomy is common surgical procedure that is cut and perforated only in the cortical bone but not the medullary bone. This procedure reduces bone resistance and increased rate of tooth movement. Kole[116] was the first one who tried corticotomy in orthodontic tooth movement. Within 6 to 12 months, tooth movements were completed. After that, this technique was used by other researches to correct open bite treatments.[117,118, 119]

In 2001 Wilcko[120] indicated that the accelerated orthodontic tooth movement could not be occurred because bony block movement as followed by Kole.[116] This situation presented bone remodeling only the surgical site. It was called Regional Acceleratory Phenomenon (RAP). After that, he developed the procedure that was termed Accelerated Osteogenic Orthodontics (AOO) and periodontal accelerated osteogenic orthodontics. Also, this technique was completed by adding grafting material to enhance bone healing.

This technique showed stability and an improved retention after postoperative,[121] however further studies are required to be confirmed. The disadvantages are invasive painful and the accelerated tooth movement only 3 to 4 months after that the time was decreased to the same level as the controls.[13, 122, 123]

4.1.2 Distraction osteogenesis

The concept of distraction osteogenesis was about studies[124] of limb lengthening. The technique is divided into distraction of PDL and the dentoalveolar bone. Later, this concept was received to use in the accelerated tooth movement which came from surgical treatments of craniofacial skeletal dysplasia.

In the accelerated canine distraction, when the first premolar extraction interseptal bone on the distal side of canine was undermined then, the resistance of distal bone on the pressure site was decreased. Tooth movement was easier and faster.[19] So, it occurred in the initial phases of tooth movement.[125]

The interseptal bone was thickness about 1 to 1.5 mm in distal side of the canine after extracted first premolar and the round bur was used to deep the socket along the length of the canine. The canine was retracted with intraoral devices. It indicated that full retraction of canine 6-7 mm into the premolar socket was spent time about 3 weeks to complete movement.[19]

In addition, accelerated canine distraction with cut dentoalveolar bone was similar to the distraction of PDL, by increased osteotomies performed at the vestibule.[20-22, 126]

Although, all studies indicated that were not significant root resorption and could accelerate orthodontic tooth movement. Nevertheless, the result showed some inconsistencies about vitality test of the retracted canines. Liou[19] indicated that 9 out of 26 teeth found positive vitality, while Sukurica[126] showed that 7 out of 20 had positive vitality after retracted canine about 6 months. Therefore, the results were controversial.

4.1.3 Piezocision

The Piezocision technique was firstly done by Dibart.[127] The procedure consisted of incision on the buccal gingiva and followed with Piezo surgical knife to the buccal cortex.[128] And periodontal tissue did not damaged by this technique. Another advantage indicated that it could be used with Invisalign, that enhanced more aesthetic appearance and decreased treatment time followed by Keser.[129] Piezocision is a hopeful accelerated tooth movement method due to the advantages on the periodontal, aesthetic, and orthodontic aspects.

4.2 Assisted Device to accelerate tooth movement

4.2.1 Low-level laser therapy (LLLT)

Surgery, diagnostics and therapeutic application with laser has been used since 1960.[130] In the orthodontic treatment brought LLLT to utilize for several treatments for instance reduced pain after adjustment[131] or traumatic ulcers treatment. The interaction of electromagnetic and light directly or indirectly with tissue could stimulate the therapeutic effects of LLLT.

In clinical and experimental studies indicated that effects of LLLT could improve would healing when applied on the injured tissues due to rapid epithelization, increased vascularization, collagen synthesis and activated fibroblast activity.[132] In addition, LLLT could stimulate bone healing, strengthened bone tissue during fracture in animal models[133] and increased maximum bone tolerance. Then, the initial phase of bone regeneration, LLLT could repair process of osteopenic fracture[134] and promoted periodontal regeneration and osteointegration of dental implants.[135]

LLLT could stimulate accelerated tooth movement with good quality bone in the mid-palatal suture expansion of rats[136] and increased production of osteoclasts.[137] Moreover, Kim et al. indicated LLLT combined with mechanical forces could activate osteoprotegerin (OPG), RANKL, and receptor activator of nuclear factor kappa-B (RANK) expression in rats.[135] Study of Fujita et al. suggested that LLLT excited rate of tooth movement through RANK and RANKL interaction pathway in an early stage.[138] Cruz et al. studied LLLT in young subjects revealed that it could speed up teeth movement and decreased whole treatment time.[16] While, Limpanichkul et al. proved that LLLT could not increase tooth movement in adult patient because of low energy density to express stimulatory effect.[139]

4.2.2 Electrical current

Direct electric current is another method to approach orthodontic tooth movement. Electrical current method was studied only in animals. The anode (7V) was applied at the pressure sites, while cathode (7 V) was attached at the tension sites. Therefore, the electrical could generate local responses and accelerated bone remodeling.[140] Several studies were successful due to electrodes could attached close to the tested tooth. However, character of the devices and the source of electricity were difficult to be clinically examined. Several researches used enzymes and glucose as fuel to improve biocatalytic fuel cells to create intraoral electricity.[141, 142] Further development of this techniques was needed to study in the clinical protocol.

4.2.3 The vibratory stimulation

The vibration used in dentistry is a low magnitude and high frequency vibration that used in orthodontic tooth movement to accelerate orthodontic tooth movement. It has been hypothesized that the vibration may accelerate bone remodeling through mechanical stimuli on the bone cells and periodontal ligament cells, etc.[44, 53, 143, 144]

5. Vibration

5.1 Fundamental of vibration

Mechanical vibration is mechanical movement that is oscillated by external force. It can be divided into 2 types i.e. 1. Free vibration is a movement system that is continuously activated at natural frequency without external force. 2. Forced vibration is made by continuous external force. The system will oscillate at the frequency of external force, if the external force activates the object that consists of the natural frequency of that object. It will create the resonance vibration and may be harmful to that system. For instance, the building is vibrated while the earthquake occurs.[145]

The characteristic movement of vibration can determine 2 patterns that is 1. Linear motion is a movement in straight direction. The position of the object can be defined with the displacement vector. 2. Angular motion is a movement of changes in angular displacement. It can specify the position of object with angular velocity or omega (**ω**)**.** In addition, direction of angular velocity can calculate with trigonometry ($X^2+Y^2=L^2$).[145]

Simple harmonic motion or simple wave

The mathematic was used to define the simplest type of wave as $a(t) = A \sin(2\pi f(t))$ where $a(t)$ is the acceleration (m/s^2) at time t. And the amplitude is A. A frequency is f cycles per second (unit = hertz, Hz) (Fig 1). If the frequency increases, the period of wave will decrease. In addition, the frequency can show in the term of radians per second (**ω**) that $\omega = 2\pi f$.[145]

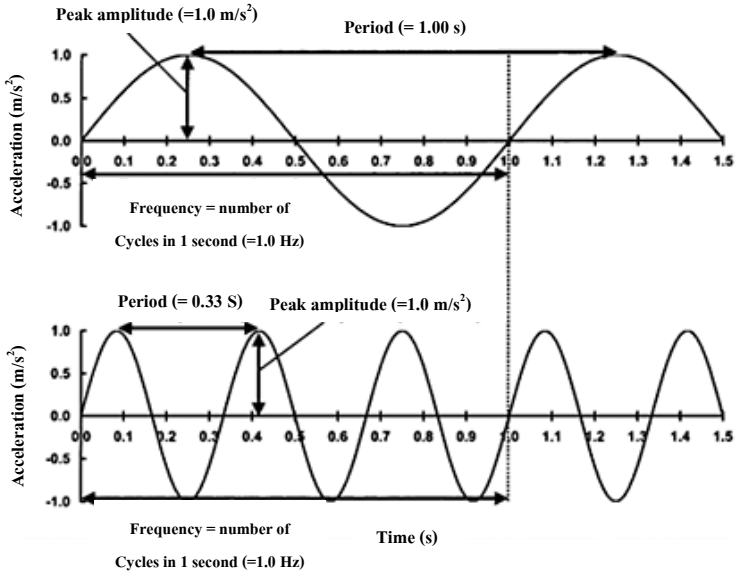


Figure 1. Showed Basic sine wave for the description of frequency at 1 Hz and 3Hz, amplitude 1 m/s^2

Vibration signal composes of displacement, velocity and acceleration that cannot truly separate. We consider the movement of the wave that moves up and down. The maximum vertical displacement appears at the top of the wave that is consistent with zero velocity. The extreme velocity appears while rising of the wave or falling of the wave (positive or negative). The minimum vertical displacement happens at the bottom of the wave. And it returns to zero velocity again. It has also related to cyclic acceleration and deceleration because of constant changing of velocity. For any wave motions, the displacement, velocity, and acceleration do not move coincidence in each other; indeed, the inverse relationship of displacement and acceleration can find in the sine wave.[145] (Fig 2)

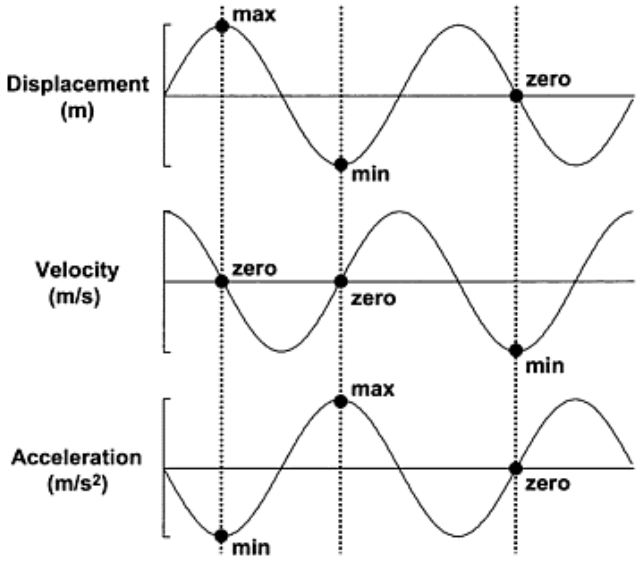


Figure 2. Showed top and bottom of sine wave for displacement, velocity, and acceleration

Resonance vibration

The wave movement will occur as per a single unit, If the structure of wave is wavered very low frequencies. While high frequencies, the vibration is separated from the structure because the vibration is limited to the position of application. Between zones of high and low frequencies, the stimulus is based for comparison when the system response will be raised to the maximum level. This is called the resonance. Resonance frequency, and complex structures can find in all of systems or structures. And they have expressed more than one. So, the method prevents the effects of resonance frequency that create a point of failure. That is a damping technique in the structure.[145] (Fig 3)

Generally, humans have naturally highly damped although resonance frequencies are still clearly apparent. It means that if the vibration exposes to the individuals, both magnitude and frequency of stimulus will affect their response.[145]

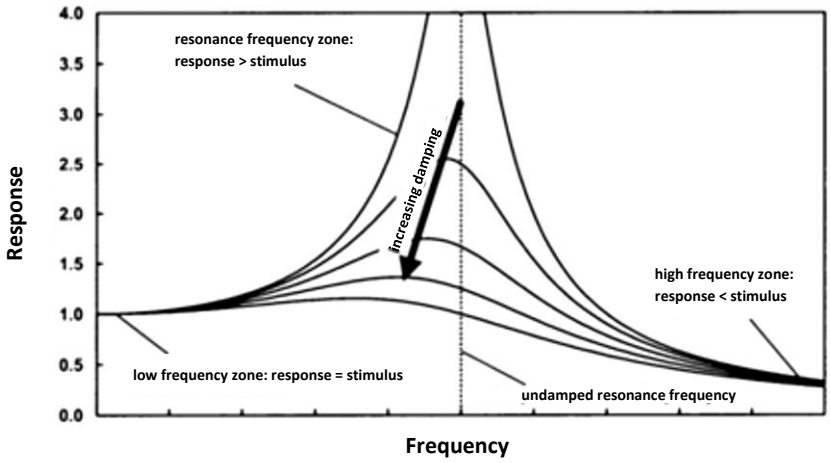


Figure 3. Showed the response of the systems to vibration. At low frequencies zone, the response and the stimulus are the same; the resonance frequency zone the response system is higher than the stimulus; at high frequencies zone, the response of the system is lower than the stimulus. As increased damping, the peak of response was decreased

5.2 Application in health science of vibration (clinical research) and possible mechanism

In the past, mechanical stimuli were used in the skeletal structure. The mechanical stimuli have a number of characters; for instance, static and dynamic which depended on magnitude and frequency. There were a number of researches which conducted in order to study the effect of mechanical stimuli to skeletal structure by performing treatment in case of low bone mass density or osteoporosis. Therefore, they could explain mechanism of bone response to the treatment.

Mechanical stimuli for therapeutic use

Vibration known as high frequency, low magnitude stimulation can be defined as a mechanical stimulus of an oscillatory motion.

The key descriptors of vibration include:

1. Frequency (measured in Hz; it indicates the number of movements up and down of wave cycles per second)

2. Amplitude (the extent of the oscillatory motion, measured in mm)

3. The direction of the vibration movement

In the previous studies, when human had exercised, the high load physical activity has a more osteogenic effect than low load physical activity.[146, 147] If bone is immobilized, bone density will be reduced.[148] Later, Hert and colleges[149, 150] tested the serial reaction of bone to mechanical stimuli. They found that dynamic loading could stimulate osteogenic effect on bone. But there are 2 limitations of the measurement: 1. it could not be measured while objects were moving. 2. It could not be measured while object was loading. In 1970, rosette strain gauge was invented by Lanyon and Smith[151] for measuring the strain in the bone. The disadvantage of rosette strain gauge is it could not measure specific parameter. So, several studies showed 5 parameters of mechanical signal;

1. Static or dynamic loading: from study of Lanyon and Rubin[152] compared the osteogenic effect of dynamic loading and static loading. The pins were fixed on the ulna bone and connect to the helical spring to release the static loading and dynamic loading. The result indicated that bone mass increased about 40% in dynamic loading bone and found thinner cortical plate combined with porosity in static loading and disuse bone which corresponded to the study of Hert and Liskova.[149]

2. The direction of loading (Axial or torsional loading) found that bone response was activated when load was applied in the ulna model. The response depended on the manner of applied load. Axial loading could promote cellular activity by increasing bone turnover. However, torsional loading inhibited bone resorption without area loss and change of the pores. This indicated that the cells can recognize the different loading that effect to bone mass, but in different ways.[153]

3. Strain duration: Rubin and Lanyon[154] said that osteogenesis effect can occur in the short period of cycle loading. The ulna models were loaded at different cycles (unload, 4, 36, 360 or 1,800) daily 0.5 Hz in six weeks. It demonstrated that the unloaded group showed no change in two weeks but gradually decreased to 88% of normal bone. At four 0.5 Hz (8 second) can maintain bone mass and prevent osteoporosis. Thirty-six 0.5 Hz (72 second) induced bone deposition gradually and reach a peak at 28 days and 360, 1800 cycles did not induce an additional change in bone characteristic than 36 cycles. It concluded that sensitivity of bone response was induced at low strain cycles.

4. Strain magnitude: Rubin and Lanyon[155] investigated the effect of strain magnitude on bone mass. Thirty turkeys were tested at 8 weeks. The different magnitude at

500, 1000, 1500, 2000, 3000 and 4000 were applied and fixed frequency at 1 Hz duration 100s. The experiment indicated that strain less than 1000 microstrains resulted in bone loss while strain over 1000 microstrains were osteogenic effect on periosteal and endosteal surface only but at 1000 microstrains bone was maintain. The study indicated that bone did not adapt to peak strain, but it adapted to the distribution of strain.

5. Strain frequency: McLeod and Rubin 1992 studied in the effect of vary frequencies between 1 Hz and 60 Hz 10 minutes on ulna model and the strain-maintained bone mass were recorded. At 1 Hz (600 cycles) required strain greater than 700 microstrains to maintain bone mass, 30 Hz (18,000 cycles), strain 400 microstrains could maintain bone mass and 60 Hz (36,000 cycles), strain 270 microstrains was required to stabilize bone mass. So, all above studies demonstrated that bone was affected by strain magnitude than cycle number. This result indicated temporal characteristics of strain signal and the frequency was ultimately imperative.

Later, there were many studies about using vibration to maintain bone mass density in post- menopausal (PMP) and disable patients, reduced bone fracture from osteoporosis and reduced osteoporosis in PMP woman. The study of Rubin et al. about the abilities of vibration signals (low magnitude high frequency) to anabolic activities in disuse. They found that mechanical stimulation 90 Hz 0.25g 10 min/day of proximal tibia rats increased bone formation rate (BFR) (97%) when compared with disuse group. This study shows that mechanical stimulation (low magnitude high frequency) increased anabolic activity and useful to long term space flight, bone loss, bed rest and immobilization due to paralysis.[39] The study of Oxfund et al. about the effect of vibration to bone mass, bone strength and muscles mass in ovariectomized (OVX) rats by vibration at 17 Hz 0.5g, 30 Hz 1.5g, 45 Hz 3g 30 min/day found that 45 Hz increased rate of periosteal bone formation and inhibition of resorption and decreased maximum bending stress and compression stress. And all groups did not affect to muscle mass. So, the possible advantages of vibration are to preserve bone of OVX animals.[156] The study of Ward et al. about the effect of low level mechanical loading 90 Hz 0.3g for 10 min/day in children with disabling conditions found that in the intervention group changed in proximal tibia volumetric trabecular bone mass density (BMD) (6.27mg/ml) when compared with non-intervention group (-9.45 mg/ml). It means that low magnitude high frequency can stimulate anabolic effect in children bone and it may be possible to stimulate activity of muscles in disabled children.[31] The study of Rubin et al. about potential of low magnitude high

frequency (30 Hz 0.3g less than 30 min/day) on the prevention of bone loss in postmenopausal women found that bone loss in femur and spine can be inhibited by applied vibration when compared with placebo (non-vibration).[28] This study showed the result as same as the study of Verschueren et al. they revealed that the high frequency at 35-40 Hz 2.28-5.09g increased BFR, bone strength and muscles strength of the hip when compared with non-vibration group.[157] The study of Gusi et al. about effect of vibrational platform 12.6 Hz, 3 cm amplitude compared walking exercise to bone mass density BMD in postmenopausal woman (PMP). They found that the vibration platform increased BMD 4.3% of femoral neck compared to walking exercise. But both of them maintained in the lumbar spine BMD.[158]

5.2.1 Whole-body vibration

The experimental application of whole-body vibration on the skeleton has been studied in animals and humans. Results suggest that whole-body vibration may be effective therapy when bone maintenance or anabolism is required such as mobility impaired patients, decreased bone density and surgical healing. (Table 1 and 2)

Table 1. Showed previous studies of Whole-body vibration in animal

Author	Hz	Population	Duration	Intensity	Outcome
Rubin et al. 2001[39]	90	Female Dawlay rats (6-8 months)	10 mins/day (4 weeks)	0.25 g	↑ Bone formation rate (BFR) and Mineralized surface (MS)/ bone surface (BS)
Christiansen and silva et al. 2006[159]	45	Male mice (7 months)	15 mins/day, (5 weeks)	0.1 g	↑ Trabecular bone Bone volume (BV)/total bone volume (TV)
Xie et al. 2006[160]	50-70	Female mice (2 months)	15 mins/day (3 weeks)	0.3 g	↓ Osteoclast activity, ↑ BFR
Castillo et al.2006[26]	20	C57BL/6 mice (12 weeks)	3 days/week (4 weeks)	2 N	↔ No effect of vibration on periosteal bone formation in the ulna
Garman et al.2007[27]	45	Adult femaleC57BL/6 mice (19 weeks)	10 mins/day (3 weeks)	0.3 g, 0.6 g	0.3 g: ↑ 88% trabecular BFR/BS, ↑ MS/BS (64%); ↑ 0.6 g: ↑ 66% BFR/BS, ↑ 22% MS/BS, ↑ 8% epiphyseal cortical area and thickness
Xie et al. 2008[161]	45	BALB/cByJ mice (8 wks)	15mins/day, 5days/week (6 weeks)	0.3 g	↑ Proximal tibial metaphysis: MS/BS Cortical bone: ↑ BV, periosteal bone area, bone marrow area, cortical area

Table 2. Showed previous studies of Whole-body vibration in human

Author	Hz	Population	Duration	Intensity	Outcome
Verschueren et al. 2004[157]	35-40	PMP (58-74yrs)	30 mins, 3times/wk	2.28-5.09g	↑ Bone mass density (BMD) (0.93%) ↑ Muscle strength (15-16%)
Ward et al. 2004[31]	90	Pubertal disable child (Pre and Post)	10 mins/d, 5 times/wk	0.3g	↑ BMD proximal tibia (6%) ↔ Cortical BMD, muscle parameter
Rubin et al. 2004[28]	30	PMP (57 yrs)	20 mins/d	0.2g	↔ BMD hip, lumbar spine ↑ Spine BMD
Iwamoto et al.2005[162]	20	Osteoporosis PMP (55-88 yrs)	4 mins, 1 time/wk	0.7-4.2 mm	↔ BMD hip, lumbar spine Marker of bone remodeling ↓ Chronic back pain
Gilsanz et al.2006[30]	30	Low-BMD females w fractures (15-20 yrs)	10 mins/d	0.3g	↑ Cancellous vertebral BV/TV ↑ Femoral cortical bone
Gusi et al. 2006[158]	12.5	PMP (66 yrs)	3times/wk WBV vs walking	3 mm	↑ BMD femoral neck (4.3%) ↔ BMD lumbar spine

Mechanisms of signaling bone transduction are not clearly explained. Bone may response to mechanical loading and transmit the signal in lacuna-canalicular network. Osteocytes might be sensors of the bone and transduce mechanical signal to the target.[163, 164] Bone is porous and contains with fluid and osteocyte that embed in the lacunar space. When the mechanical stimuli are loaded. they press the interstitial fluid in the lacunar space through the cell process and surrounding cells towards the Harversian canal and produce fluid shear stress to the osteocyte in lacunar space.[164] The fluid shear stress enhanced the osteocyte to release the Prostaglandin E2 and nitric oxide which might control resorptive activity.[165] In addition, fluid shear stress stimulated to the osteocyte and inhibited the formation of osteoclasts. This indicates role of osteocyte in controlling the osteoclast formation after applied mechanical loading.[166]

5.2.2 Orthodontic vibration

Low-magnitude, high-frequency vibration has been applied experimentally in the field of orthodontics focusing on increasing the rate of orthodontic tooth movement.

Roberts et al.[167] considered to the limiting factor of rate orthodontic tooth movement in bone resorption at the PDL surface. A maximum rate of molar translation in the maxilla is approximately 2mm per month for space closure in a rapidly growing child or 1mm per month for space closure in a non-growing adult.[168] Clinically and experimentally there have been many attempts to increase the rate of orthodontic tooth movement, including the decreased friction in the orthodontic appliances,[169] increasing adjunctive medical or hormonal therapies (local or systemic)[170] and more recently adding surgical corticotomy techniques.[120]

Vibration has minimizing side effects when compare to medicinal treatments. It also was proven to safe and decrease complication substitute that enhances bone remodeling in the medical field[30, 171] and dental field.[172] In addition, the vibration may rouse the orthodontic tooth movement rate through the signal pass from periodontal ligament to the target cell for the proliferation and differentiation. And the vibration stimulates osteogenesis by increasing periodontal ligament cells. Study of Zhang et al. about the effect of vibration to human periodontal ligament stem cells found that protein Runt-related transcription factors 2 (RUNX2) and Osterix (OSX) had an influence on the differentiation and activation of osteoblasts.[143] The study of Xue et al. found that the vibration can stimulate the alveolar bone remodeling by expression of Hepatocyte growth factor/RUNX2/bone morphogenetic protein 2 (BMP-2) and RANKL in rat orthodontic tooth movement model.[144] Furthermore, it may also

directly activate the osteocyte in lacunar-canalicular network that makes a motion of nucleus osteocyte and sends the information for the differentiation.[163, 164] All of these effects may increase inflammatory cytokines, the number of osteoclasts and growth hormone for migration of blood vessel that stimulate osteoclastogenesis process.[173]

In addition, the study of Olsen et al. about archwire vibration and stick-slip behavior bracket-archwire found that at the high and medium amplitude (190 and 150 mV) (50 mV = 0.08 mm) can decrease frictional resistance but the low amplitude (100 mV) cannot overcome the frictional resistance. And the variations of frequency in archwire are not significant to decrease frictional resistance.[174] The study of Yu-Jin seo et al. about the effect of tooth displacement and vibration on frictional force and stick-slip phenomenon in conventional bracket and 0.018-inch Cu-NiTi found that the vibration at 30 Hz. 0.25 N did not decrease Static frictional force (SFF), kinetic frictional force (KFF) or stick-slip phenomenon (SSP) amplitude.[175] But when passive and active self-ligating brackets were used the vibration can move diverse tooth displacement condition and significant reduced SFF, KFF, SSP amplitude and increased SSP frequency.[176] These are different due to types of bracket and methods of ligation of archwire on the bracket and the amount of tooth displacement. However, true mechanisms of vibration are uncertainly determined.

Previous study's results on vibratory stimulation for accelerating tooth movement were controversy. Some studies showed that tooth movement can be accelerated but others did not. Because protocol to use vibration and phenomenon of vibration may not be clearly defined. The number of studies is limited and contradictory. No one has elucidated the ideal frequency for optimal response if such a frequency even exists. (Table 3)

Table 3. Showed studies of Vibratory stimulation for accelerated tooth movement

Author	Hz	Population	Duration	Intensity	Outcome
Darendeliler et al.2007[24]	30	Wistar rats (7 weeks) pulped electromagnetic fields (PEMF)	8 hours	-	↑ Rate of tooth movement PEMF-induced vibration
Nishimura et al. 2008[44]	60	Male wistar rats (6 weeks)	8 mins on (0,7,15 d)	-	↑ Rate of tooth movement, RANKL, osteoclast
Liu et al. 2009	4	Mice (20 weeks)	5 mins	20 μm	↑ Rate of tooth movement (40%)
Kau CH et al.2010[177]	30	Humans (20.3 yrs)	20 mins/day	20 g (0.2N)	↑ Rate of tooth movement, ↔ Root resorption
Mile P et al. 2012[50]	111	Humans (11-15 yrs)	20 mins/day	6.1 g (0.06N)	↔ Rate of tooth movement (Control vs Vibration)
Kalajzic Z et al. 2013[178]	30	Sprague-Dawley rats (7 weeks)	10 mins, twice a week	0.1-0. 4N	↓ Orthodontic tooth movement, bone volume fraction, osteoclast
Leethanakul C et al. 2015[53]	125	Humans (19-25 yrs)	15 mins per day for 2 months	-	↑ Rate of tooth movement and secretion of IL1-beta
Woodhouse NR et al. 2015[179]	30	Humans (means 14 yrs)	20 mins/day	20g (0.2N)	↔ Rate of tooth movement in initial tooth alignment or redece treatment time

Table 3. (CONTINUED)

Author	Hz	Population	Duration	Intensity	Outcome
Pavlin D et al. 2015[54]	30	Humans (14-40 yrs)	20mins/day	25g (0.25N)	↑ Rate of tooth movement
Yadav et al. 2015[47]	5, 10, 20	CD1 mice (age 12 weeks)	15mins/day every 3 days for 2 weeks	1cN	↔ Rate of molar mesialization
Mile et al. 2016[49]	30	Human (mean age 12.8 yrs)	20mins/day	25g (0.25N)	↔ Rate of tooth movement in initial tooth alignment
Mile et al. 2018[180]	30	Human (mean age 12.8 yrs)	20mins/day	25g (0.25N)	↔ Rate of space closure (mandibular canine distalization)
DiBiase et al. 2018[52]	30	Human (age less than 20 yrs)	20mins/day	25g (0.25N)	↔ Rate of space closure (mandibular canine distalization)

From the literature reviews show that the frequency of whole-body vibration and orthodontic vibration are controversy. Although the frequency of whole-body vibration is different in each study however, the outcome of frequencies indicated that it can increase bone formation. Orthodontic vibration presents different frequencies like a whole-body vibration. Nevertheless, the effects of frequencies to bone response for accelerated tooth movement are controversy. It is not the same as whole-body vibration. Because structure of alveolar is not the same long bone, it stimulates to periodontal ligament instead of bone cells and orthodontic force is a factor that different from whole-body vibration. So, in this study tries to find the appropriate frequency to stimulate bone remodeling for accelerated tooth movement. Frequency at 30 and 60 Hz are chosen because the study of vibration at 30 Hz showed results that are controversy. So, the frequency at 30 Hz was chosen to investigate the

true effect. For the frequency at 60 Hz showed the result that it can accelerate tooth movement when combining with orthodontic force, however, the experiment was designed in the animal model. It cannot imply that effect of frequency at 60 Hz to human because the structure, growth, bone remodeling and cell response are different from human. In the part of frequency, more than 100 Hz was not chosen because the vibration is quite high, and it occurs numbness from vibration and annoying voice that it may induce to decrease compliance.

6. Nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) receptor activators

The RANKL consists of six exons which localized on human chromosome 13q14.[181] The spans of RANKL was approximately 36 kb of genomic DNA. The RANKL gene (hRANKL1) transcripts of 2,271 bp due to un translated regions of 5 and 3 ends mRNA transcription.[181] While, hRANKL2 and hRANKL3 absent the N-terminus intracellular domain which making a soluble forms. In addition, Both forms are still translated at downstream in-frame start codons.[182] Furthermore, C-terminal extracellular domains of all three isoforms are the same.

The tumor necrosis factor (TNF) family is consisted of RANKL member. The mouse RANKL has a similar sequence homologous to 87% of human protein.[183] TNF-related apoptosis-inducing ligand, TRAIL,[183] and Fas ligand,[181] have a relationship to the human RANKL sequence about ~34% and ~28% sequence homology, respectively. RANKL has 317 amino acid, 45 kDa membrane-associated protein.[183] The soluble proteins have a 31 kDa and 39.5 kDa that 31 kDa soluble protein act as proteolytic cleavage and 39.5 kDa soluble protein will be raised when hRANKL3 isoform will be expressed.[184] The biological activity is exhibited by the soluble forms. Type II transmembrane protein is the hRANKL1 which comprises of 20 amino acid between residues Met48 and Phe67. Then, C-terminal extracellular domain is a region (Phe68 to Gln163), which contains Ile140 and Ala145 amino acid sites that have a two-potential processing. In addition, the active receptor binding site composed of two N-linked glycosylation sites at 171–173 and 198–200 amino acids and a TNF-family homologous domain (from Pro164 to Val313).

Osteoblasts can produce RANKL and OPG to regulate osteoclast formation and function which depend on their expression. Tumor necrosis factor receptor associated factors (TRAFs) is moved to the intercellular domain by binding of RANKL to RANK on osteoclast precursors. TRAFs 1–3, 5 and 6 are complicated with several intracellular signaling

pathways such as activation of NF-kappa B, the Akt/protein kinase B pathway and mitogen-activated kinase pathways (via JNK, Erk and p38).[185] These pathways control osteoclast precursors accumulation, differentiation into mature osteoclasts, and their activation and survival. In contrary, binding of OPG and RANKL is an antagonist to its interaction with RANK and decreased numbers and function of osteoclasts.

The level of bone resorption was determined by the role of RANKL, OPG and RANK system that expressed in transgenic mice. RANKL and RANK mice was not demonstrated osteoporotic because of absence of osteoclasts. While, OPG mice were increased numbers of osteoclasts that were severely osteoporotic.[186] Therefore, relative of RANK, RANKL and OPG are determined bone resorption under normal and disease conditions. In humans, RANKL mutations lead to the congenital diseases but cannot identify. Nevertheless, mutations in OPG and RANK can indirectly enhance RANKL signaling, leading to produce increased bone remodeling of Juvenile Paget's disease[187] or familial expansile osteolysis[188] respectively.

The response of RANKL/OPG to vibration

The results of increasing bone mass density from vibration were shown in table 1 and 2. And the vibration used in orthodontic treatment was shown in table 3. The results from table 3 are still controversial. However, the mechanism of vibration that reacts to bone cells is unclear. The studies have shown the biomarkers (RANKL, OPG) of osteoclast for differentiation, survival, and function, which indicate metabolism of bone.[44, 189-192] In bone remodeling, vary proliferation markers are presented. Especially, RANK, RANKL and OPG play an important role to osteoresorptive effect which produced by many hormones and cytokines.[193] This process is occurred by osteoblast cell release the RANKL from activation of cytokines to combine with RANK on osteoclast precursor cell for expression resorptive effect. After binding, the osteoclast precursor cells differentiate to mature osteoclast. After that OPG is produced by osteoblast which is a decoy receptor. OPG has a role to compete for RANK-RANKL binding to inhibit osteoclast differentiation, repression of activation of osteoclast and stimulation of apoptosis. Therefore, balance of RANK-RANKL-OPG binding is controlling bone remodeling. In addition, RANKL and OPG specific to receptor that presents on the surface of osteoclast precursor cell. It can tell us to increase or decrease resorption or formation process. Furthermore, the number of osteoclasts formation and their activity is identified by the RANKL

and OPG ratio. In addition, it can regulate the rate of bone remodeling. However, RANKL/OPG ratio is the markers chosen to define the rate of bone resorption and formation.

The study of Karin Pichler et al.[189] On vibration to RANKL, OPG in osteoporosis mice found that vibration can decrease the RANKL and increase the OPG in 12 weeks. Bergstrom et al.[190] studied the effect of RANKL/OPG in postmenopausal women with physical training. They found that OPG increased and RANKL decreased, so bone resorption was inhibited. Chung et al.[191] studied the effect of vibration 35 Hz 0.3g 20 minutes/days to enhance bone formation during bone healing in rat. They found that RANKL/OPG system increased. Lau et al.[194] study the effect of low-magnitude, high-frequency vibration in regulation of osteoclasts via osteocytes indicated that vibration could stimulate osteocytes response at the transcription level. And Maximum response of COX-2 level was at 90 Hz about 334% and RANKL decreased at 60 Hz. about 55%. In the condition medium, found that the amount of sRANKL lower in the vibration groups and decreased osteoclast formation and resorption. So, osteocyte responds to vibration and producing the soluble factors that affected to decrease osteoclast formation. The study of Wu et al.[195] about the effect of low magnitude high frequency vibration on osteoclast differentiation in vitro found that low magnitude high frequency vibration decreased the amount of RANKL-induced TRAP-positive multinucleated cells and mRNA expression showed that decreased TRAP and Cathepsin-K in vibration group at 45 Hz (0.3g) for 15 minutes. Moreover, the vibration could inhibit expression of c-Fos in the RANKL-treated RAW264.7 cells. Therefore, the RANKL-induce osteoclast differentiation of RAW264.7 cells was inhibited by low magnitude high frequency vibration that showed the anabolic effects of low magnitude high frequency vibration to the bone. In addition, Hou et al.[192] studied micromechanical vibration-enhanced osteogenic response of osteoblasts. They found that the vibration at different amplitude at 40 Hz 30 minutes/day reduced the expression of RANKL and increased the expression of OPG. The study of Nishimura et al.[44] on vibration combined with orthodontic force can enhance the rate of orthodontic tooth movement via expression of RANKL in mice. They found that the expression of RANKL increased in vibration group and the rate of tooth movement was increased. However, it can be concluded that the studies on the expression of RANKL/OPG to vibration are still unclear, especially the mechanobiology.

Gap of knowledge

1. No consensus about the clinical effect of vibratory stimuli on the rate of canine distalization.

2. No study on the biological response to the combination of orthodontic force and vibration especially, RANKL/OPG ratio in human.

Objective

General objective

To investigate the effect of varying vibratory frequencies on the rate of canine distalization.

Specific objective

1. To study the outcome of different frequencies on the secretion of RANKL/OPG during canine distalization.

Research questions

1. Do different frequencies of vibration affect to rate of canine distalization?
2. Are there any differences in the secretion of RANKL/OPG when orthodontic force is applied with and without vibratory stimuli?
3. Do different frequencies vibratory affect the secretion of RANKL/OPG ratio during canine distalization?

Hypothesis

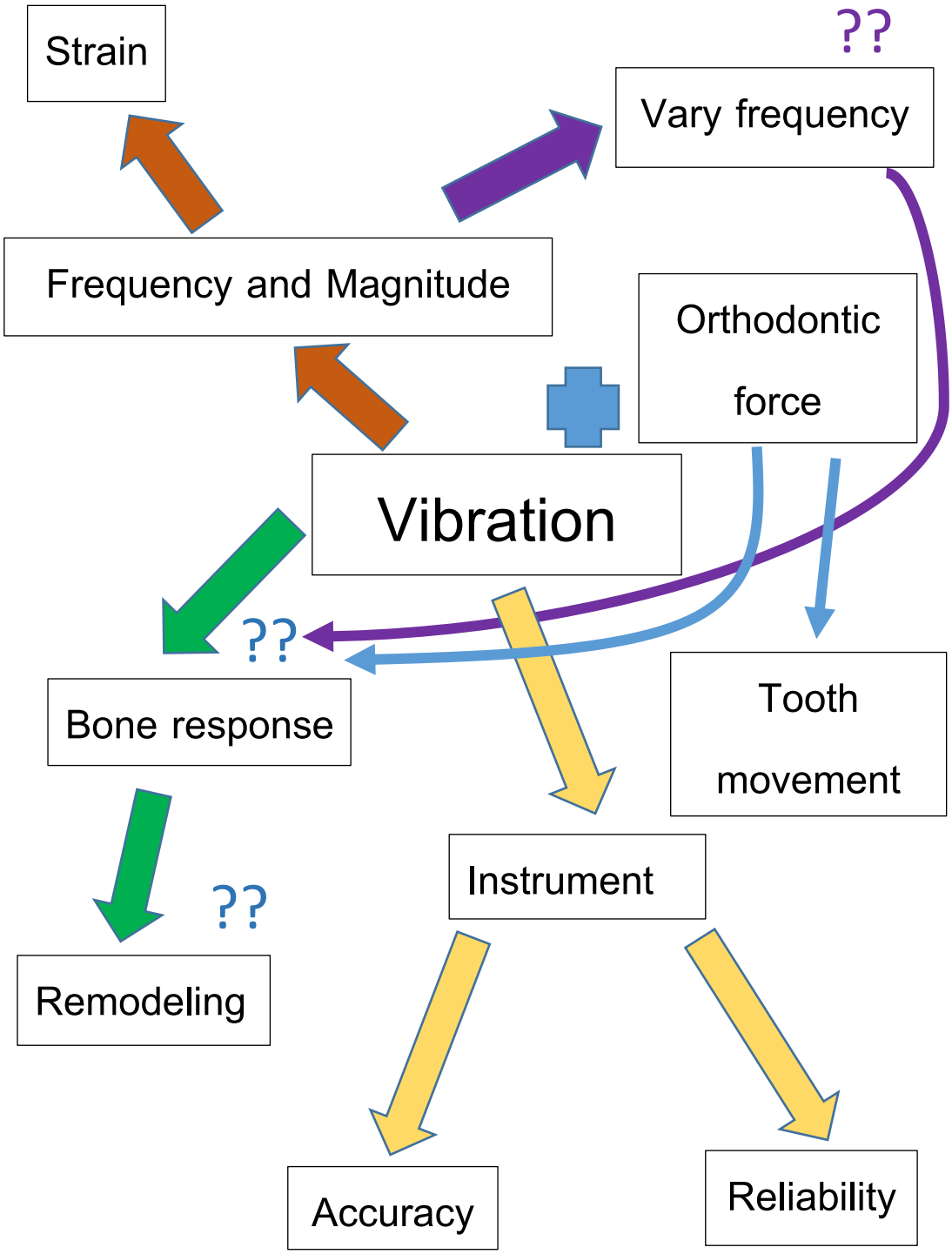
1. The rate of canine distalization is not significantly different from varied vibratory frequency groups.
2. No significant difference in the secretion of RANKL/OPG when orthodontic force is applied with or without vibratory stimuli.
3. The secretion of RANKL/OPG during canine distalization is not different between varied vibratory frequency groups.

Significance of study:

The vibratory stimulation combined with conventional orthodontic treatment has superior advantages over orthodontic treatment only which are

1. The combination of vibratory stimulation in orthodontic treatment may lead to the change of RANKL/OPG ratio, which possibly alters the bone response stimulated from different vibratory frequencies.
2. The suitable vibratory frequencies for the acceleration of tooth movement can be defined.

Conceptual framework



CHAPTER 2

RESEARCH METHODOLOGY

Materials and Methods

The Ethics Committee on Human Research of the Faculty of Dentistry, Prince of Songkla University approved this randomized controlled clinical trial (RCT) for clinical research in human (ethical approval *No.* EC5805-21-9-HR*)* and the Thai Clinical Trial Registry Database was used to register the data of research *(TCTR20170707004)*.

Subjects

The G*power program version 3.1 was used to calculated the sample size on the outcomes of three different methods on the canine distalization rate.[196] the mean differences among the first method, second method, and third method were 0.35, 0.58 and 0.81 mm/month, respectively (difference in standard deviations = 0.34 mm/month, significance level = 0.05, power = 0.90). A minimum subject was required in each group that was 15 subjects per group. Then, the subjects were gathered until 20 per group to make for dropout. Therefore, A total subjects in this study were involved about 60 subjects.

Participants were recruited from Orthodontic Clinic, Faculty of Dentistry, Prince of Songkla University during August 2015 and December 2017. The inclusion criteria were 18 to 25-year-old subject who were planned for upper first bicuspid extraction and canine distalization with good general and oral health, no signs of gingival inflammation or periodontal disease, and no history of anti-inflammatory, immunosuppressive, bisphosphonates or steroid drug use within the 6 months before beginning treatment. Subjects who were unable to continue treatment or presented periodontal problems during the study were excluded. All participants who met the inclusion criteria and were informed of the purpose and nature if study had signed an informed consent before participating in the study.

Vibratory devices

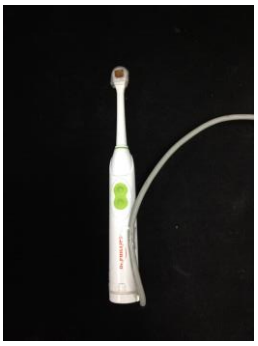
The vibratory stimuli were generated from custom made vibrators which were modified from electric toothbrush (Fig. 4) (Dr. Phillips® FRESH-EX, Tego Dental & Chemical Co., Ltd, Bangkok, Thailand). The brush was removed from each device. The tip was covered with 2 mm-thick ethylene-vinyl-acetate (BIOPLAST®, Scheu™-Dental, Iserlohn, Germany) (Fig. 5A, 5B,

and 5C). The power of these devices was used with alternating electric current (220 V) (Fig. 4). The magnitude of vibratory device was generated 0.1 g (g-force, where g = earth's gravitational field, acceleration at 9.81 m/s^2). The different frequencies were set and confirmed, calibrated, standardized with a vibration sensor (MTI-2100 Fotonic^{TM} sensor instruments; MTI Instruments, Inc. Albany, NY, USA) (Fig.6A) and an oscilloscope. (Fig. 6B) The accuracy and reliability of each device were confirmed by operating the devices for 20 min, five times. The average percentage coefficients of variation of these vibratory devices were 0.06 ± 0.01 and 0.03 ± 0.01, respectively for the 30 Hz and 60 Hz devices. While, Intra-class correlation extended from 0.993 and 0.995. Thus, the vibratory devices can be considered to have produced the determined frequency with high accuracy and reliability.

Figure 4. Showed the electrical circuit of the vibration

A

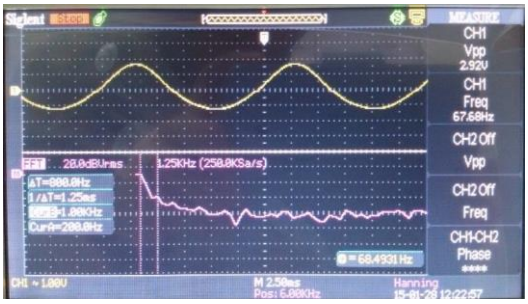
B



C

Figure 5. (A) Showed the Ethylene-Vinyl-Acetate or BIOPLAST®. (B) Showed the vacuum machine (MINISTAR SCHEU®). (C) Showed the vibratory after modified the tip of electrical toothbrush

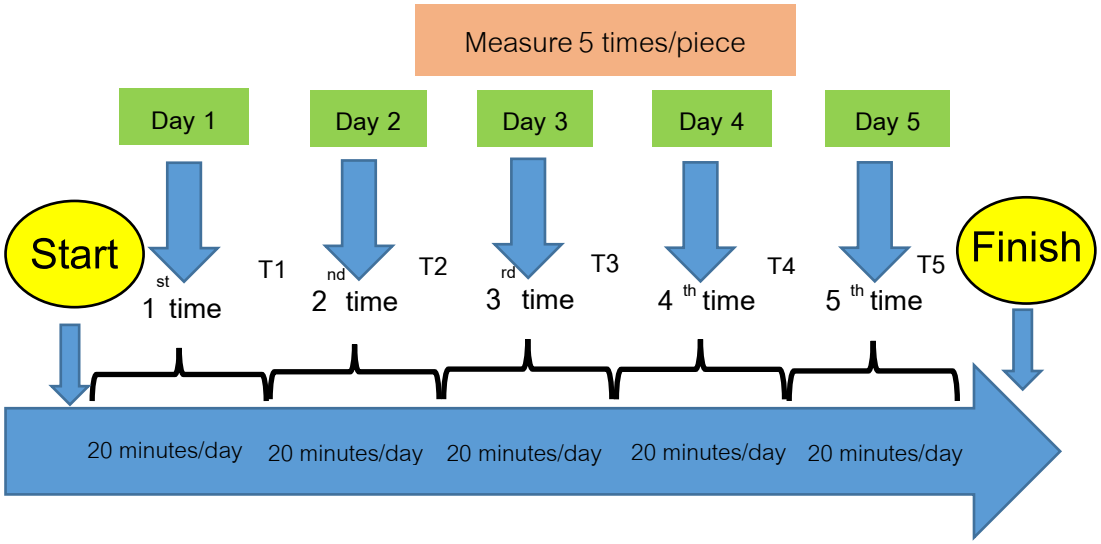
A



B

Figure 6. (A) showed the MTI-2100 Fotonic™ sensor to measure the accuracy and reliability of the vibrator. (B) showed the oscilloscope for interpretation of the signal from MTI-2100 Fotonic™ sensor

The schedule of reliable test



Randomization

Randomization for this three-arm parallel RCT with a 1:1:1 allocation ratio was accomplished following the CONSORT 2010 guidelines (Fig. 7). A card shuffling method[197] was used to randomly allocate the 60 subjects into three groups: (1) canine distalization + 30 Hz vibratory stimuli (30 Hz group), (2) canine distalization + 60 Hz vibratory stimuli (60 Hz group), or (3) canine distalization only (control group).

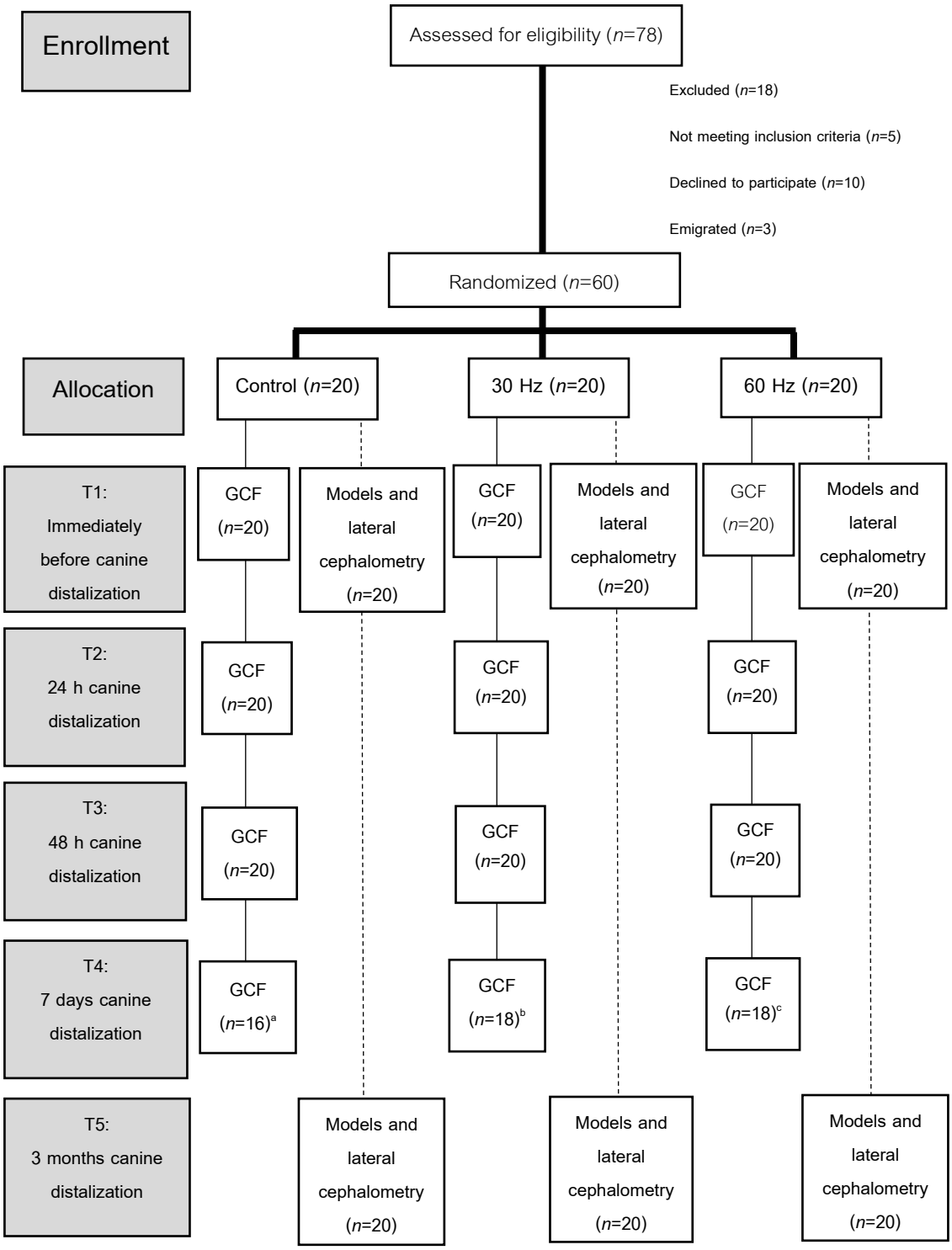


Figure 7. CONSORT diagram of subject randomization and data collection and analysis. GCF = gingival crevicular fluid, [a] One, two, and one subject(s) in the control group were absent on days 3, 4, and 5 respectively, [b] One and one subject in the 30 Hz group were absent on days 3 and 6, respectively, [c] One and one subject in the 60 Hz group were absent on days 3 and 4, respectively. Solid lines indicate the per-protocol analysis; dashed/broken lines indicate the intention-to-treat analysis.

Intervention protocol

All subjects were treated by the first investigator. Two weeks after the first premolar teeth were extracted, 0.022-inch slot pre-adjusted edgewise appliances (Roth™ system; Ormco, CA, USA) were bonded from the right second molars to the left second molars. All subjects received the same sequence of archwire from 0.012-inch, 0.014-inch, 0.016-inch, 0.016 × 0.016-inch, 0.016 × 0.022-inch nickel-titanium (NiTi) archwires, to 0.016 × 0.022-inch stainless steel (SS) archwires. When the teeth were well aligned and levelled, the four incisors were tied together using a 0.001-inch SS ligature wire. All posterior teeth on each side were also colligated together. The maxillary arch was maintained with a 0.016 × 0.022-inch SS archwire for 4 weeks before the start of canine distalization.

To distalize the maxillary canine, 60 g of force was used and generated from a NiTi closed-coil spring. The spring was clasped to the hooks of maxillary second molar buccal tube and the canine bracket. The spring was reactivated every four weeks. A force gauge (Ortho Organizers, Inc., San Marcos, CA, USA) was used at each visit to confirm the force remained constant throughout the study period.

A card shuffling method was also applied to randomly define the side investigated (left or right) for each subject. During the first part of the study (days 1–7 after initiation of distalization), the first investigator recalled all subjects to attend every day at about the same time for application of vibratory stimuli and data collection. For the subjects in experimental group (the 30 Hz and 60 Hz groups), the archwire on maxillary arch was removed and the head of the vibratory device was gently touched at the mesio-incisal line angle of the designated canine and turned on for 20 minutes (Fig. 8). In the control group (received orthodontic treatment only), The subjects were received the same procedure again, except the vibratory device was only touched and not turned on. The analysis of this part was used with a per-protocol analysis. Some subjects missed a single appointment. They were excluded from this part of the study analysis.

For the second part of the study (days 8 to 90), intention-to-treat analysis was applied. Every subject was taught how to use the vibratory stimuli device by themselves and was given a vibratory device with the allocated frequency. The subjects were received a daily reminder message via the subjects' mobile phones to encourage compliance. Each subject was also given a log book to self-record the start and finish times of the vibratory stimulus intervention each day. Reminder messages via mobile phone and log books have been

claimed to be effective reminding tools in several medical compliance studies.[198, 199] During this stage, the first investigator recalled subjects every 4 weeks for activation NiTi closed-coil spring and data collection.

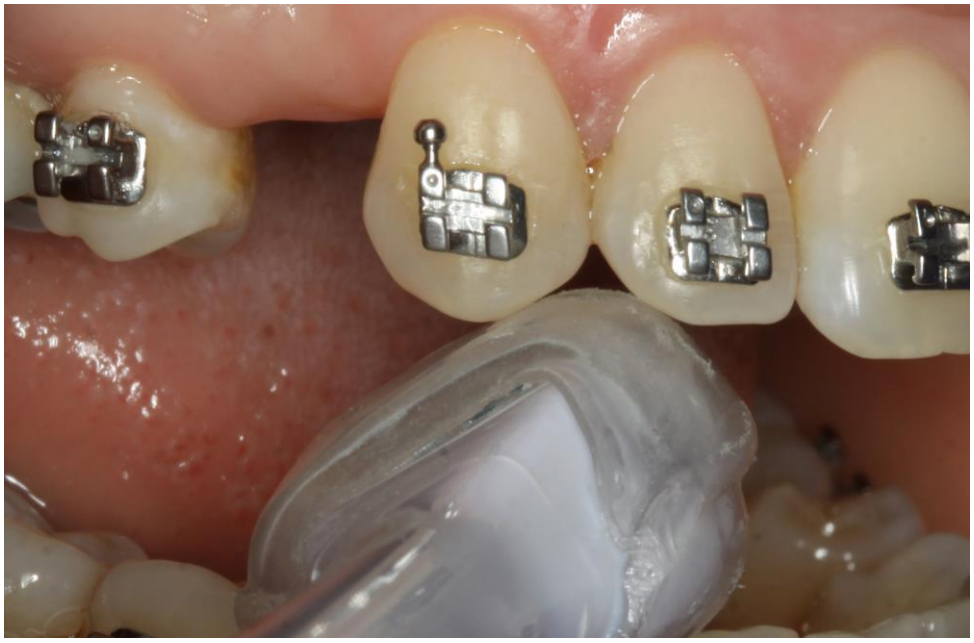


Figure 8. Applied the vibratory device using light touch on the mesio-incisal line angle of the canine

Data collection

The first investigator collected the data of this study at five timepoints (Fig. 7): immediately before canine distalization (T1); 24 h (T2); 48 h (T3); 7 days (T4); and 3 months after started canine distalization (T5).

Gingival crevicular fluid collection and analysis

Clinical preparation started with the teeth from which GCF was collected (T1, T2, T3 and T4) were isolated according to the method of Offenbacher et al.[200] At the mesial and distal sides of the maxillary canines, PerioPaper strips (Oraflow Inc., Smithtown, NY, USA) were put in the gingival sulcus to collect GCF. (Fig. 9) The PerioPaper strips were quantified the GCF volume with Periotron 8000 (Siemens Medical Systems, Inc., Iselin, NJ, USA). The commercial RANKL and OPG enzyme-linked immunosorbent assay kits (Duoset® ELISA Development Kits; R&D Systems, Minneapolis, Minnesota, USA) was used to analyse the concentrations of RANKL and OPG. The GCF samples were pulled together. The protease

inhibitor (60 µl; SantaCruz™ Biotechnology; Santa Cruz, CA, USA) and sterile phosphate-buffered saline (60 µl; pH 7.4) were used to dilute the GCF samples and the samples were shaken gently for 20 min at 4 °C, and centrifuged (3,000 g at 4 °C for 15 min). After that, the samples were analysed in duplicate samples to define the concentrations of RANKL and OPG.

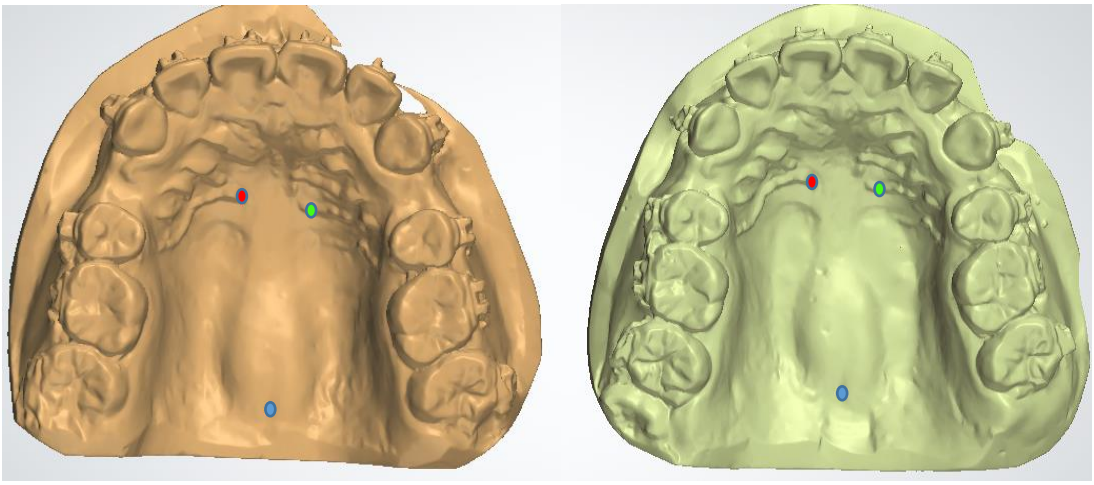
Figure 9. Showed gingival crevicular fluid collection

Study models

Alginate impressions (ALGINoplast® Kulzer GmbH; Leipziger StraBe 263450, Hanau, Germany) were taken at T1 and T5. Impressions were poured with orthodontic stone to fabricate study models. All models were scanned using an Orthodontic 3D Scanner (R700 model, 3Shape, Ivoclar Vivadent, Inc., Copenhagen, Denmark). The digital models were superimposed with Ortho Analyzer software (3Shape Ivoclar Vivadent Inc.) based on a modified version of the technique designated by Jang et al.[201] by three points on the model at the right and left medial third palatal rugae and fovea palatine, as shown in Figure 10.

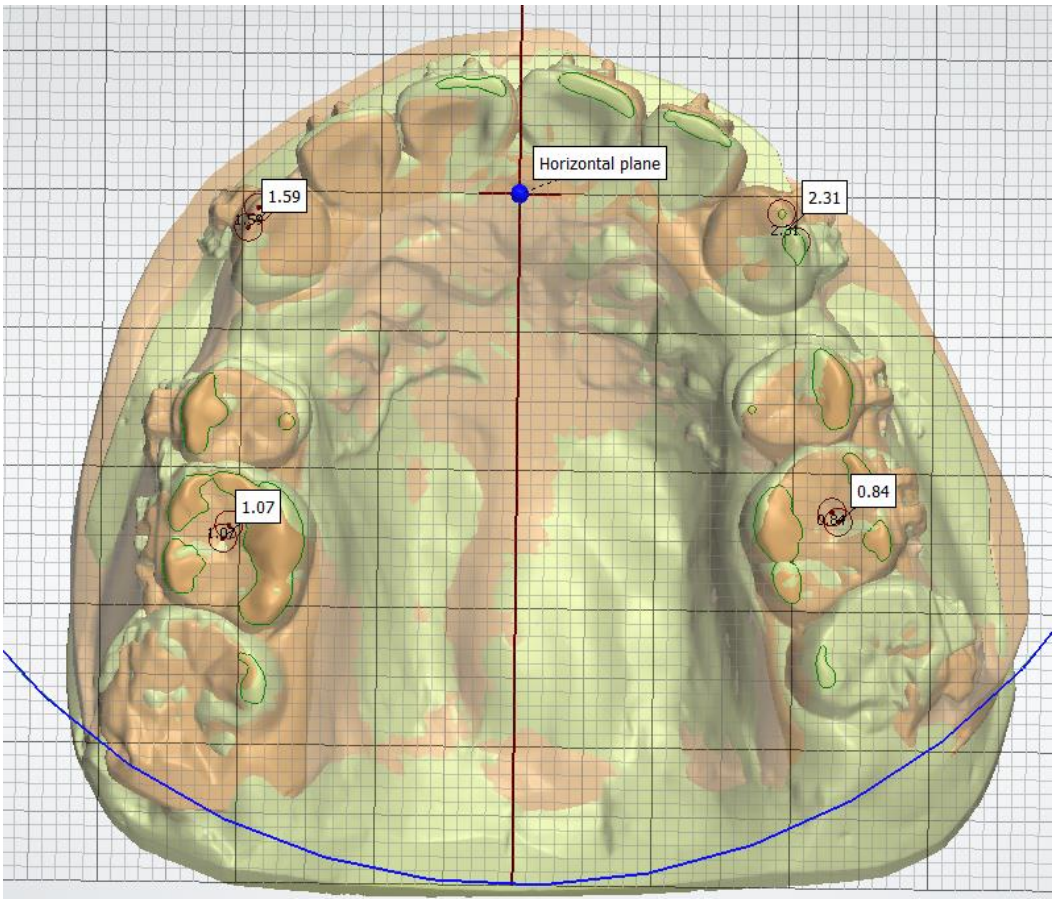
The rates of canine and molar movement were investigated from distance from T1 to T5 divided by the 3-months treatment time, in which the distance was measured from the change of canine cusp tip and central pit of molar. (Fig. 11).

The rotation of canine was examined from the angle formed with two reference lines. The first line was constructed from the middle of the incisive papilla to the midpoint between the left and right fovea palatine. The second line passed through the mesio-labio-incisal point angle to disto-labio-incisal point angle of the canine (Fig. 12, 13). Angular changes between two timepoints were compared.



Before canine retraction model Final canine retraction model

Figure 10. Showed three reference points for superimposition



Model superimposition

Figure 11. Showed model superimposition and distance of canine and molar movement

Before canine retraction model

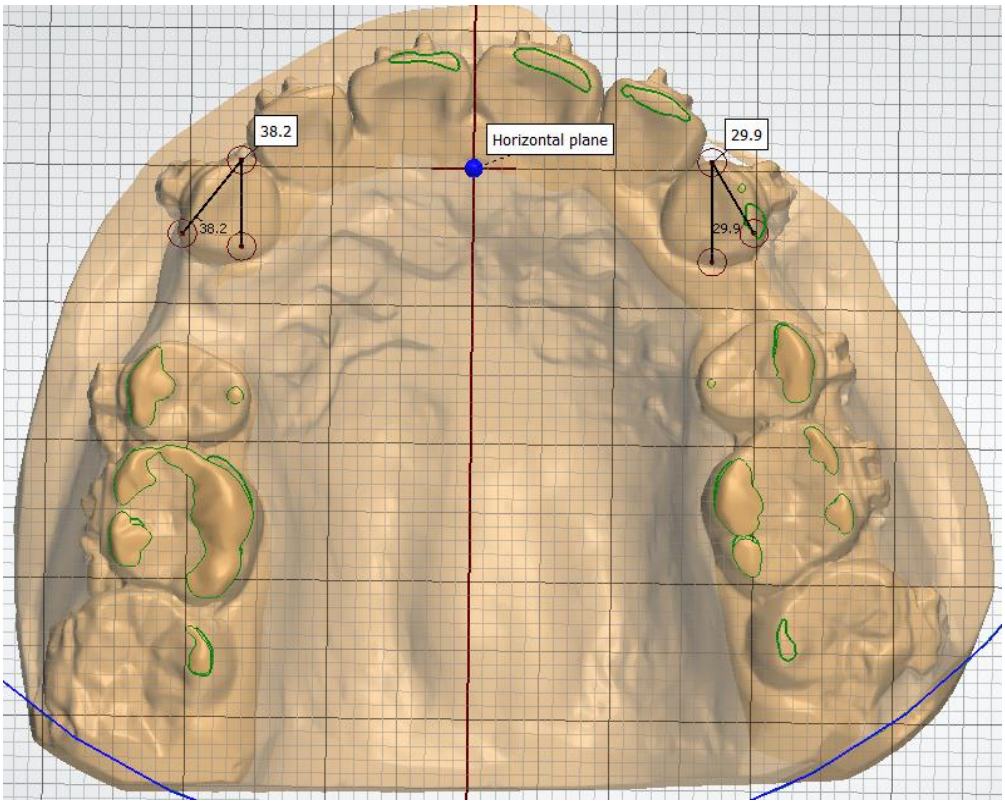


Figure 12. Showed the angle of canine before canine movement

Final canine retraction model

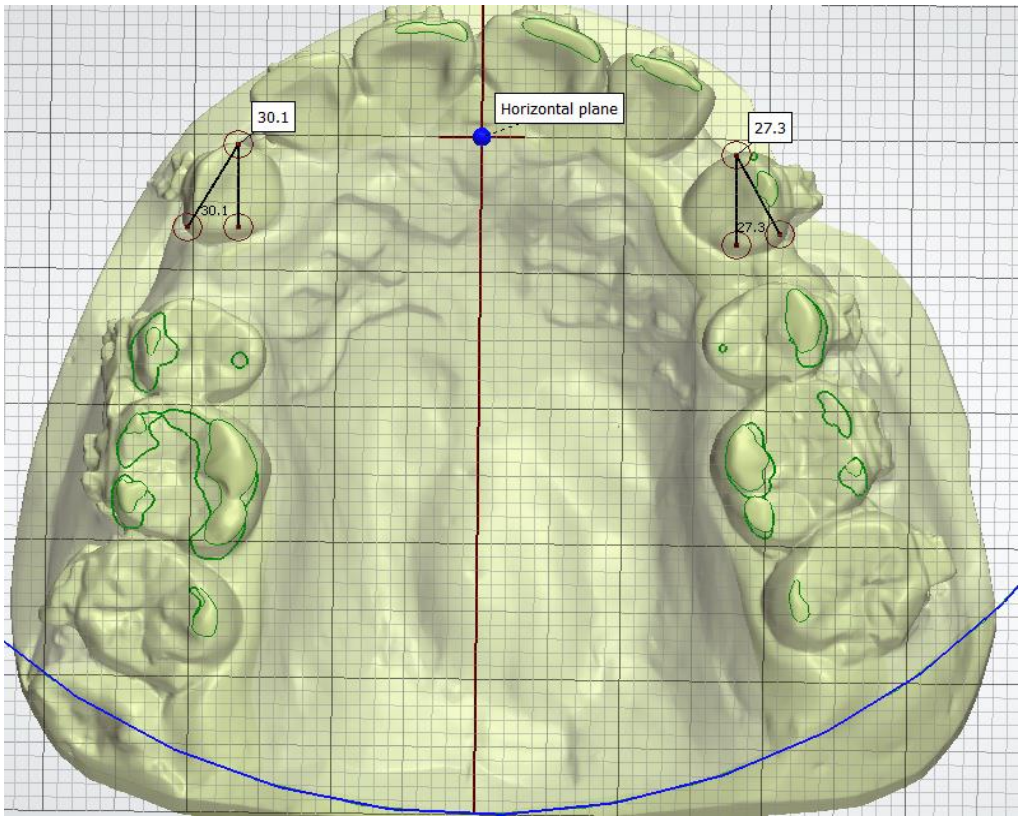


Figure 13. Showed the angle of canine after finished canine movement

Lateral cephalograms

Lateral cephalograms were taken at T1 and T5 with the same machine (Gendex GXDP-700™; Hatfield, PA, USA) using reference jigs made of 0.016" × 0.022" SS wire inserted on the canine bracket and second molar tube.

The canine and second molar angulation changes were calculated from the angles formed with the palatal plane (anterior nasal spine [ANS]-posterior nasal spine [PNS]) and the reference jigs on each tooth. The angulation changes between T1 and T5 were assessed (Fig. 14).

Blinding

The first investigator and subjects were aware of the received vibratory stimuli or not. However, the subjects who received the vibratory stimuli were blinded to the frequency of vibratory stimuli that they were randomized to receive. All records were labelled and rearranged to conceal the co-researchers who made data analysis to the group allocations.

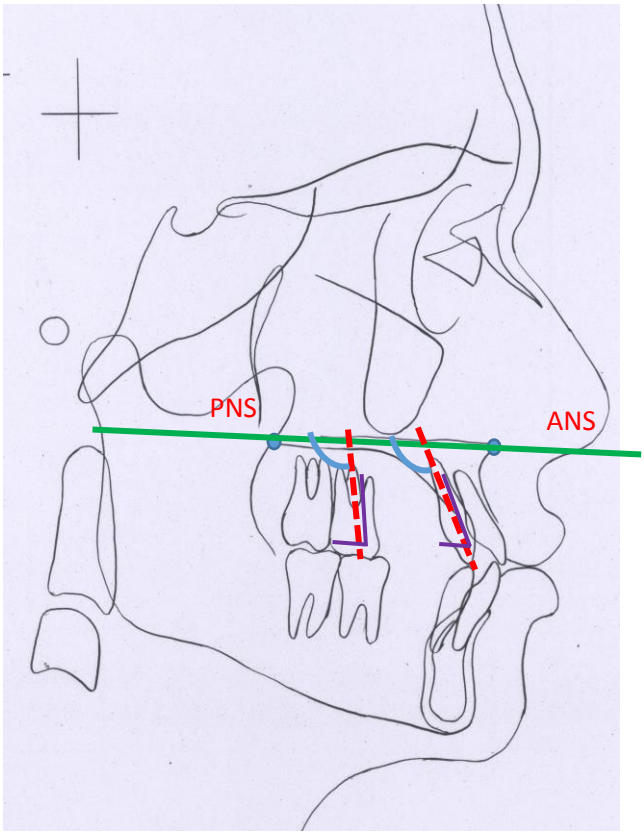


Figure 14. Showed reference points, planes and angulations of cephalometric analysis

Statistical analysis

Some outcome variables indicated that were abnormally distributed by Shapiro-Wilk tests. Consequently, the appropriated statistics analysis was applied. Initial cephalometric values, age, and sex were analysed with One-way analysis of variance (ANOVA) and the Chi-square test to compare between groups. The analysis of GCF and all clinical parameters within-group changes between timepoints and between-group changes at each timepoint were examined with Kruskal–Wallis tests followed by Dunn's tests for post-hoc comparisons. The random errors and the reliability measurement were assessed by Dahlberg's formula[202] and intraclass correlation coefficients (ICCs), for all cephalometric and model data. Ten measurements records were randomly selected and remeasured again by the same investigator after an interval of 4 weeks. The significant level was established at 0.05 for all investigation. Method error did not exceed 0.5 mm and 0.5° for linear variables and angular variables measurement, respectively. The reliability of repeatability of measurement was acceptable at ICC = 0.86-0.99.

CHAPTER 3

RESULTS

The experimental protocol is presented in the CONSORT diagram in Figure 7. The sixty subjects recruited were randomly allocated to three groups in equal numbers. In the first part study (per-protocol analysis), eight subjects missed one or more appointment in 7 days experiment. They were excluded from the analysis. Subsequent,18 subjects in the 30 Hz group, 18 subjects in the 60 Hz group, and 16 subjects in the control group. For the second part study (intention-to-treat analysis), eights subjects were excluded from the first part. They were recruited to the second part for analysis at the T1 and T5 data collection.

The subject's characteristics in each group are shown in Table 4. Age, sex or initial cephalometric values before treatment were not significantly presented difference between groups. ($P > 0.05$).

Within group comparisons exhibited that the concentration of RANKL at T1 was significantly inferior than at T2, T3, or T4 ($P < 0.000$, $P < 0.001$, and $P < 0.003$, respectively) on the compression side in the control group. While, the concentrations of RANKL and OPG and the RANKL/OPG ratio were not significant difference between groups at any time point on the tension and compression sides ($P > 0.05$). Similarly, the between-group comparisons of RANKL and OPG and the RANKL/OPG ratio concentrations were not significantly different at any time point on both sides ($P > 0.05$; Table 5; Fig. 15A and 15B)

With respects to the measurement of model and lateral cephalometric, the canine movement rates (0.82, 0.87, and 0.83 mm/month; $P > 0.05$), the molar movement rates (less than 0.2 mm/month in every group ($P > 0.05$), canine rotation and tipping and molar tipping ($P > 0.05$; Table 6) were not significant difference between the 30 Hz, 60 Hz, and control groups.

Table 4. Comparison of the initial characteristics of the patients in the 30 Hz and 60 Hz vibration intervention groups and control group

Variable	30 Hz (*n* = 20) (Mean ± SD)	60 Hz (*n* = 20) (Mean ± SD)	Control (*n* = 20) (Mean ± SD)	*P*-value
Sex (M:F)	3:17	5:15	5:15	0.675[a]
Age (years)	21.6±2	22.1±2.5	20.9±1.7	0.189[b]
SN-GoMe (degrees)	34.9±4.1	34.4±5.1	33.4±4.2	0.574[b]
FMA (degrees)	26.1±3.1	25.8±4.4	24.7±4	0.438[b]
Occl-SN (degrees)	17.2±3.1	16.3±4.4	15.7±3.9	0.433[b]
SNA (degrees)	83.9±3.1	84.1±2.8	84.1±3.6	0.978[b]
SNB (degrees)	79.9±3.3	79.7±3.1	80.9±3.7	0.475[b]
ANB (degrees)	4.0±2.3	4.4±2.4	3.4±1.8	0.321[b]
Overjet (mm.)	3.9±1.2	4.3±1.3	3.6±1.2	0.187[b]
Overbite (mm.)	2.8±1.1	2.8±1.2	2.8±1.3	0.989[b]

[a] Chi-Square analysis

[b] One-way analysis of variance (ANOVA)

Table 5. Concentrations of RANKL and OPG and RANKL/OPG ratio for the 30 Hz and 60 Hz vibration intervention groups and control group

Timepoint	Group	RANKL (pg/mL) Median (IQR)		OPG (pg/mL) Median (IQR)		RANKL/OPG Median (IQR)	
		Compression	Tension	Compression	Tension	Compression	Tension
T1	30 Hz (*n* = 18)	144.96 (62.64, 365.51)	184.37 (152.02, 270.99)	272.36 (206.53, 546.95)	241.11 (136.26, 430.61)	0.54 (0.18, 1.06)	0.81 (0.62, 1.02)
	60 Hz (*n* = 18)	208.85 (154.18, 409.97)	192.67 (84.70, 379.65)	351.26 (114.28, 1083.32)	226.63 (166.07, 273.96)	0.62 (0.40, 1.87)	0.88 (0.32, 1.39)
	Control (*n* = 16)	189.48 (132.20, 278.21)	189.02 (131.80, 291.52)	340.99 (228.19, 465.30)	190.32 (130.49, 328.51)	0.45 (0.33, 0.83)	0.61 (0.50, 2.23)
	P-value[a]	0.226	0.984	0.866	0.744	0.396	0.967
T2	30 Hz (*n* = 18)	311.31 (177.20, 543.85)	207.57 (130.67, 334.22)	316.86 (237.74, 534.54)	284.25 (168.39, 391.17)	0.86 (0.51, 1.27)	0.68 (0.48, 1.15)
	60 Hz (*n* = 18)	376.04 (224.75, 607.32)	227.81 (122.62, 334.88)	390.89 (258.01, 1203.47)	331.78 (192.97, 591.50)	0.84 (0.38, 1.44)	0.62 (0.34, 1.39)
	Control (*n* = 16)	450.34 (239.34, 817.51)	290.68 (127.94, 647.14)	300.58 (154.09, 599.68)	366.04 (215.19, 525.35)	1.31 (0.43, 3.25)	0.76 (0.30, 2.20)
	P-value[a]	0.347	0.534	0.388	0.205	0.534	0.927

Table 5. (CONTINUED)

Timepoint	Group	RANKL (pg/mL) Median (IQR)		OPG (pg/mL) Median (IQR)		RANKL/OPG Median (IQR)	
		Compression	Tension	Compression	Tension	Compression	Tension
T3	30 Hz (*n* = 18)	269.69 (168.91, 490.96)	230.24 (75.28, 522.55)	341.27 (178.61, 483.30)	292.28 (209.52, 427.69)	0.84 (0.60, 1.45)	0.64 (0.43-1.10)
	60 Hz (*n* = 18)	340.96 (185.43, 430.53)	262.81 (178.21, 398.96)	449.57 (293.87, 1025.73)	348.55 (290.49, 895.61)	0.76 (0.46, 1.24)	0.62 (0.34, 1.11)
	Control (*n* = 16)	425.34 (314.23, 570.54)	307.78 (257.86, 596.09)	550.99 (234.80, 901.44)	372.62 (208.75, 637.83)	1.03 (0.39, 1.54)	0.84 (0.54, 1.73)
	P-value[a]	0.182	0.186	0.238	0.386	0.853	0.407
T4	30 Hz (*n* = 18)	287.84 (210.02, 705.63)	291.12 (199.98, 419.84)	293.79 (233.55, 642.47)	274.04 (148.38, 444.40)	0.89 (0.61, 1.24)	0.63 (0.33, 2.15)
	60 Hz (*n* = 18)	356.48 (173.41, 874.98)	269.96 (86.86, 436.06)	438.60 (169.65, 1306.86)	339.36 (271.96, 669.72)	0.68 (0.37, 3.22)	0.53 (0.30, 1.16)
	Control (*n* = 16)	428.63 (204.60, 635.58)	263.74 (195.64, 418.43)	508.06 (280.49, 804.44)	398.72 (185.68, 869.59)	0.53 (0.35, 2.53)	0.78 (0.23, 2.18)
	P-value[a]	0.929	0.834	0.466	0.229	0.889	0.612

[a] *P*-values (between groups) calculated using the Kruskal-Wallis test

Time-points measured from initiation of canine distalization. T1 = Immediately before canine distalization, T2 = 24 h canine distalization, T3 = 48 h canine distalization, T4 = 7 days canine distalization

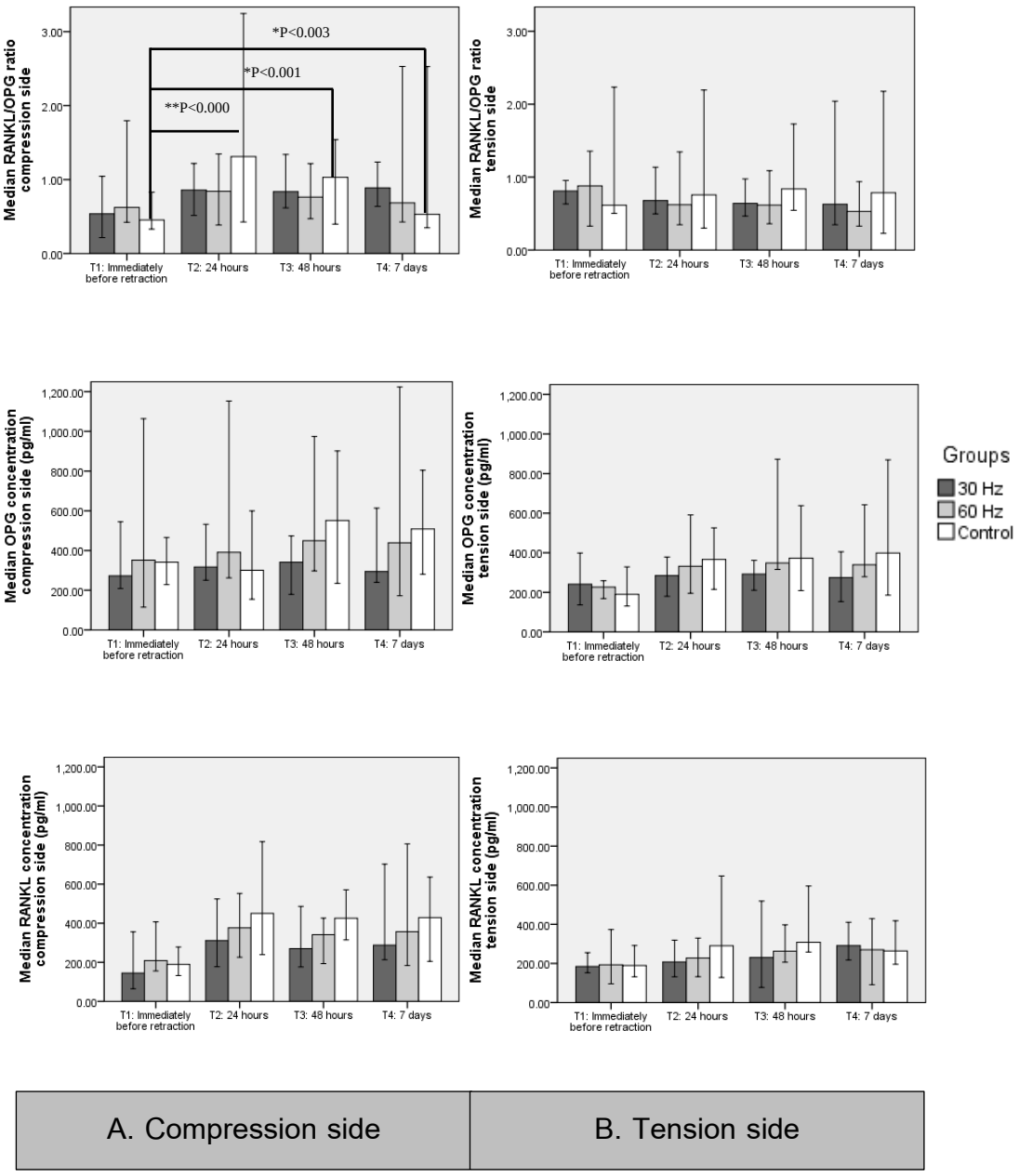


Figure 15. Concentrations of RANKL and OPG in GCF and the RANKL/OPG ratio on the (A) compression side and (B) tension side in the 30 Hz, 60 Hz, and control groups *$P < 0.005$, **$P < 0.001$, Kruskal-Wallis test and Dunn's post-hoc test

Table 6. Rates and angular changes in tooth movement for the 30 Hz and 60 Hz vibration intervention groups and control group

Variable	30 Hz (*n* = 20) Median (IQR)	60 Hz (*n* = 20) Median (IQR)	Control (*n* = 20) Median (IQR)	*P*-value[a]
Rate of canine movement (mm/month)	0.82 (0.58, 1.03)	0.87 (0.63, 1.10)	0.83 (0.71, 1.02)	0.688
Rate of molar movement(mm/month)	0.18 (0.12, 0.23)	0.19 (0.12, 0.24)	0.15 (0.10, 0.17)	0.107
Canine rotation changes (degrees)	10.0 (7.8, 13.1)	8.5 (7.3, 12.1)	10.9 (7.0, 13.2)	0.647
Canine angulation changes (degrees)	5.0 (4.0, 6.4)	5.0 (3.1, 6.9)	5.3 (4.0, 6.0)	0.748
Molar angulation changes (degrees)	2.0 (0.6, 3.9)	2.3 (1.0, 2.9)	2.0 (1.0, 2.5)	0.776

[a] Kruskal-Wallis test

CHAPTER 4

DISCUSSIONS

The ability of different frequencies of vibratory stimulus to accelerate orthodontic tooth movement has been assessed in several clinical studies.[52-55, 180] The results of these studies even those that used the same frequencies of vibration are controversial. Moreover, comparisons between studies must be made with caution due to the difference of the vibration protocols, subject characteristics, mechanics of tooth movement, and outcomes measurement.

In this study, both 30 and 60 Hz vibratory stimulus did not increase the secretion of inflammatory cytokines and accelerated the rate of canine distalization. The trivial effects of 30 Hz vibratory stimuli are in concordance with several clinical studies that investigated the ability of vibratory stimulus to alleviate dental irregularities,[48, 49, 51] but are in different results to some animal studies.[43, 44] A rat study revealed that 30 Hz vibratory stimuli significantly increased rate of molar movement (by 1.45-fold) compared to the control side.[43] When the frequencies were increased to 60 and 120 Hz, the molars moved 2.1- and 2.4-fold faster than the control teeth, respectively; 60 Hz vibration also increased the levels of RANKL released and osteoclast numbers in animal models.[43, 44] No acceleratory effect was observed in a different mouse study when the vibratory stimuli was as low as 5-20 Hz.[47] This may indicate orthodontic tooth movement may be exerted a dose-response relationship by vibration. To our knowledge, no other studies had studied the effect of 60 Hz vibration on the orthodontic tooth movement rate in humans, though a RCT studies showed 125 Hz vibration significantly accelerated canine distalization as well as IL-1β secretion.[53] This study found 30 and 60 Hz vibration had no acceleratory effects, with a lack of significant differences of RANKL and OPG concentrations between the control and experimental groups at each time point on both the compression and tension sides. These findings may indicate 30 and 60 Hz vibration applied in combination with light orthodontic force have not affected or lacked to activate increasing release of inflammatory cytokines from PDL and alveolar bone cells when compared to orthodontic force only. Further studies are desired to prove the dose-response relationship both vibratory stimuli and the orthodontic tooth movement rate in the clinical setting.

In our control group, RANKL concentrations on the compression side were significantly different at 24 h, 48 h and 7 days after initiation of distalization compared to the initial baseline concentration. The peak concentratons of RANKL were observed at 24 h, in agreement with of Nishijima et al.[102] Nevertheless, in our study, the RANKL concentration at 24 h was not significantly different from at 48 h or 7 days. These results could indicate that light continuous force may continuously activate RANKL secretion on the compression side if the force is not lost and is kept constant.[112] Note that high variation of the inflammatory cytokine concentrations is observed between samples that may be responsible for the insignificant within-group and between-group differences. The high variation in the RANKL and OPG concentrations in our study may be due to these cytokines were collected from GCF, which only permits the soluble forms of these cytokines to be analysed. Cytokine concentrations could vary depending on their ability to dissolve in GCF, which may also vary between individuals. The cytokine concentration measurement of animal studies are more exact because non-soluble membrane-bound cytokines and soluble cytokines can be determined.[203] But, membrane-bound cytokines analysis in humans study is immoral.

In the normal situation, the force from the mechanical stimuli to the teeth was moved to the PDL and the alveolar bone. Loss of mechanical stimuli leads to atrophy of periodontal ligament for instance decreased periodontal space, distortion of mechanoreceptor structure and vascular constriction[204, 205] and also cancellous bone reduction and impede cortical bone formation.[206] According to the mechanostat theory of Frost 1990,[207, 208] proposed that minimum effective strain (MES) could predict adaptation of bone changes to mechanical loading. When the mechanical loading was applied on the periodontium. The modelling and remodelling process was arisen. The modelling process was occurred on the overloading, but remodelling process was occurred on underloading. So, strains from mechanical loading for modelling process that occurred on the tension side was above 1,500-3,000 microstrains that showed increased OPG concentration and decreased RANKL concentration on the tooth movement. The mechanical strains were between 300-1,500 microstrains indicated that bone formation equalled to the resorption to maintain bone architecture. While, mechanical strains above 3,000 microstrains was appeared on the compression side implied that RANKL concentration was increased and accelerated inflammatory process, increased microfracture and created resorption. Therefore, alveolar bone homeostasis regulated by mechanical stimuli for maintaining the alveolar bone structure and alveolar processes during existence. In

addition, the results did not show significant difference between experimental and control groups. Because the intensity of vibration could not stimulate periodontal ligament to secrete inflammatory mediators for bone resorption followed the theory's Frost mechanostat.

In contrary to the situation of periodontal diseases, the patients presented with periodontal disease indicated that inflamed periodontal tissues increased inflammatory cytokines especially RANKL lead to the formation of osteoclasts. Osteoclasts was formed the monocyte/macrophage lineage and stimulate bone resorption. Therefore, osteoclastic activity increased without bone formation to homeostasis bone structure and occurred bone loss in periodontitis patients.[209, 210] When orthodontic force was applied could arouse root resorption, pocket deepening, reduction of alveolar bone or attachment loss.[211, 212] So, RANKL concentration in periodontitis patient was found more than in the healthy patient that presence of plaque induced inflammation.

In order to precisely explore the effect of frequency, two other factors, i.e., the magnitude and duration of vibration, were controlled. We used a magnitude of 0.1 g (0.0007 N), which is very low compared to the protocols used in other human studies.[48-52, 54, 55, 180] Using a low magnitude of mechanical vibration ensures no significant supplementary force is provided in addition to the orthodontic force, thus the effect of varying the frequency could be determined more precisely. Moreover, the use of higher magnitudes of vibration may increase patient discomfort and increase the risk of pathogenic effects to the periodontal structure.[43] In terms of the duration of application of vibration, we followed the protocols from the previous studies and approved by the majority of previous studies to do comparisons possible. According to one animal study,[43] varying the duration of exposure to vibratory stimulus had no effect on the rate of tooth movement. It would be interesting to further investigate the effect of varied frequencies combined with varied magnitudes and durations of vibratory stimulus on the rate of orthodontic tooth movement.

This study has a number of strengths. The analysis of expression of inflammatory cytokines was conducted via per protocol analysis. Thus, patient's compliance was eliminated.[213, 214] The intention-to-treat basis was used to examine measurements of tooth movement, which reduces the accidentally overoptimistic results by exclusive of non-compliers and instead allowing some events may arise in clinical setting.[213, 214]

Given that compliance may vary between person, this study could be improved by embedding a time recorder into the vibratory stimulus device. Therefore, we may be able

to examine the effect of vibratory stimulus on the rate of orthodontic tooth movement stratified by the amount of time the patients used the device to apply vibratory stimulus.

Based on our results, the null hypothesis revealed that no significant difference in the secretion of RANKL, OPG, RANKL/OPG ratio and rate of canine distalization when orthodontic force is applied with or without vibratory stimuli was accepted. However, information from a 3 months experiment cannot be inferred to apply to the entire orthodontic treatment course. A long-term investigation is essential to precisely determine the effect of vibratory stimulus on the rate of orthodontic tooth movement.

CHAPTER 5

CONCLUSIONS

The 30 Hz and 60 Hz vibration applied with 60 grams canine distalization 20 minutes/day for 7 days did not affect the expression of RANKL, OPG and RANKL/OPG ratio. With 3-month application, the effect on the rate of canine and molar movement, canine and molar angulation and canine rotation were not found.

REFERENCES

1. Reitan K. Tissue behavior during orthodontic tooth movement. ***Am J Orthod Dentofacial Orthop*** 1960; 46(12): 881-900.
2. Krishnan V, Davidovitch Ze. Cellular, molecular, and tissue-level reactions to orthodontic force. ***Am J Orthod Dentofacial Orthop*** 2006; 129(4): 1-32.
3. Proffit WR, Fields Jr HW, Sarver DM. Contemporary orthodontics. St. Loius, Missouri, USA: Elsevier Health Sciences; 2006: 286-293.
4. Bassett CAL, Becker RO. Generation of electric potentials by bone in response to mechanical stress. ***Science*** 1962; 137(3535): 1063-1064.
5. Zengo A, Bassett C, Pawluk R, Prountzos G. In vivo bioelectric potentials in the dentoalveolar complex. ***Am J Orthod Dentofacial Orthop*** 1974; 66(2): 130-139.
6. Baumrind S. A reconsideration of the propriety of the “pressure-tension” hypothesis. ***Am J Orthod Dentofacial Orthop*** 1969; 55(1): 12-22.
7. Fink DF, Smith RJ. The duration of orthodontic treatment. ***Am J Orthod Dentofacial Orthop*** 1992; 102(1): 45-51.
8. Fisher MA, Wenger RM, Hans MG. Pretreatment characteristics associated with orthodontic treatment duration. ***Am J Orthod Dentofacial Orthop*** 2010; 137(2): 178-86.
9. Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ. Reducing white spot lesions in orthodontic populations with fluoride rinsing. ***Am J Orthod Dentofacial Orthop*** 1992; 101(5): 403-407.
10. Bishara SE, Ostby AW. White Spot Lesions: Formation, Prevention, and Treatment. ***Semin Orthod*** 2008; 14(3): 174-182.
11. Segal G, Schiffman P, Tuncay O. Meta analysis of the treatment-related factors of external apical root resorption. ***Orthod Craniofac Res*** 2004; 7(2): 71-78.
12. Pandis N, Nasika M, Polychronopoulou A, Eliades T. External apical root resorption in patients treated with conventional and self-ligating brackets. ***Am J Orthod Dentofacial Orthop*** 2008; 134(5): 646-651.
13. Aboul-Ela SMBE-D, El-Beialy AR, El-Sayed KMF, et al. Miniscrew implant-supported maxillary canine retraction with and without corticotomy-facilitated orthodontics. ***Am J Orthod Dentofacial Orthop*** 2011; 139(2): 252-259.

14. Hassan AH, Al-Fraidi AA, Al-Saeed SH. Corticotomy-assisted orthodontic treatment: review. ***Open Dent J*** 2010; 4: 159.

15. Marquezan M, Bolognese AM, de Souza Araújo MT. Effects of two low-intensity laser therapy protocols on experimental tooth movement. ***Photomed Laser Surg*** 2010; 28(6): 757-762.

16. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: A preliminary study. ***Lasers Surg Med*** 2004; 35(2): 117-120.

17. Yamaguchi M, Hayashi M, Fujita S, et al. Low-energy laser irradiation facilitates the velocity of tooth movement and the expressions of matrix metalloproteinase-9, cathepsin K, and alpha (v) beta (3) integrin in rats. ***Eur J Orthod*** 2010; 32(2): 131-139.

18. Kim D-H, Park Y-G, Kang S-G. The effects of electrical current from a micro-electrical device on tooth movement. ***Korean J Orthod*** 2008; 38(5): 337-346.

19. Liou EJ, Huang CS. Rapid canine retraction through distraction of the periodontal ligament. ***Am J Orthod Dentofacial Orthop*** 1998; 114(4): 372-382.

20. Sayin S, Bengi AO, Gürton AU, Ortakoglu K. Rapid canine distalization using distraction of the periodontal ligament: a preliminary clinical validation of the original technique. ***Angle Orthod*** 2004; 74(3): 304-315.

21. Igeri H, Kisnisci R, Bzizi N, Tüz H. Rapid canine retraction and orthodontic treatment with dentoalveolar distraction osteogenesis. ***Am J Orthod Dentofacial Orthop*** 2005; 127(5): 533-541.

22. Altug A. Dentoalveolar distraction osteogenesis for rapid orthodontic canine retraction. ***J Oral Maxillofac Surg*** 2002; 60(4): 389-394.

23. Showkatbakhsh R, Jamilian A, Showkatbakhsh M. The effect of pulsed electromagnetic fields on the acceleration of tooth movement. ***World J Orthod*** 2009; 11(4): 52-56.

24. Darendeliler MA, Zea A, Shen G, Zoellner H. Effects of pulsed electromagnetic field vibration on tooth movement induced by magnetic and mechanical forces: a preliminary study. ***Aust Dent J*** 2007; 52(4): 282-287.

25. Cowin S. Wolff's law of trabecular architecture at remodeling equilibrium. ***J Biomech Eng*** 1986; 108(1): 83-88.

26. Castillo AB, Alam I, Tanaka SM, et al. Low-amplitude, broad-frequency vibration effects on cortical bone formation in mice. ***Bone*** 2006; 39(5): 1087-1096.

27. 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(6): 732-740.

28. Rubin C, Recker R, Cullen D, et al. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: a clinical trial assessing compliance, efficacy, and safety. *J Bone Miner Res* 2004; 19(3): 343-351.

29. Rubin C, Judex S, Qin Y-X. Low-level mechanical signals and their potential as a non-pharmacological intervention for osteoporosis. *Age Ageing* 2006; 35(2): 32-36.

30. Gilsanz V, Wren TA, Sanchez M, et al. Low-Level, High-Frequency Mechanical Signals Enhance Musculoskeletal Development of Young Women With Low BMD. *J Bone Miner Res* 2006; 21(9): 1464-1474.

31. Ward K, Alsop C, Caulton J, et al. Low magnitude mechanical loading is osteogenic in children with disabling conditions. *J Bone Miner Res* 2004; 19(3): 360-369.

32. Kim IS, Song YM, Lee B, Hwang SJ. Human mesenchymal stromal cells are mechanosensitive to vibration stimuli. *J Dent Res* 2012; 91(12): 1135-1140.

33. Zhang C, Li J, Zhang L, et al. Effects of mechanical vibration on proliferation and osteogenic differentiation of human periodontal ligament stem cells. *Arch Oral Biol* 2012; 57(10): 1395-1407.

34. Zhang C, Lu Y, Zhang L, et al. Influence of different intensities of vibration on proliferation and differentiation of human periodontal ligament stem cells. *Arch Med Sci* 2015; 11(3): 638-646.

35. Shapiro E, Roeber F, Klempner L. Orthodontic movement using pulsating force-induced piezoelectricity. *Am J Orthod Dentofacial Orthop* 1979; 76(1): 59-66.

36. Nicolella DP, Moravits DE, Gale AM, Bonewald LF, Lankford J. Osteocyte lacunae tissue strain in cortical bone. J Biomech 2006; 39(9):1735-1743.

37. Uzer G, Pongkitwitoon S, Ian C, et al. Gap Junctional Communication in Osteocytes Is Amplified by Low Intensity Vibrations In Vitro. *PloS one* 2014; 9(3): e90840.

38. Weinheimer-Haus EM, Judex S, Ennis WJ, Koh TJ. Low-Intensity Vibration Improves Angiogenesis and Wound Healing in Diabetic Mice. *PloS one* 2014; 9(3): e91355.

39. Rubin C, Xu G, Judex S. The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli. *FASEB J* 2001; 15(12): 2225-2229.

40. Suzuki N, Kitamura K, Nemoto T, et al. Effect of vibration on osteoblastic and osteoclastic activities: Analysis of bone metabolism using goldfish scale as a model for bone. *Adv Space Res* 2007; 40(11): 1711-1121.

41. Benjakul S, Jitpukdeebodintra S, Leethanakul C. Effects of low magnitude high frequency mechanical vibration combined with compressive force on human periodontal ligament cells in vitro. *Eur J Orthod* 2017; 40(4): 356-363.

42. Phusuntornsakul P, Jitpukdeebodintra S, Pavasant P, Leethanakul C. Vibration enhances PGE2 , IL-6 and IL-8 expression in compressed hPDL cells via cyclooxygenase pathway. *J Periodontol* 2018; 89(9): 1131-1141.

43. Alikhani M, Alansari S, Hamidaddin MA, et al. Vibration paradox in orthodontics: Anabolic and catabolic effects. *PloS One* 2018; 13(5): e0196540.

44. Nishimura M, Chiba M, Ohashi T, 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(4): 572-583.

45. Takano-Yamamoto T, Sasaki K, Fatemeh G, et al. Synergistic acceleration of experimental tooth movement by supplementary high-frequency vibration applied with a static force in rats. *Sci Rep* 2017; 7(1): 1-14.

46. Kalajzic Z, Peluso EB, Utreja A, et al. Effect of cyclical forces on the periodontal ligament and alveolar bone remodeling during orthodontic tooth movement. *Angle Orthod* 2014; 84(2): 297-303.

47. Yadav S, Dobie T, Assefnia A, et al. Effect of low-frequency mechanical vibration on orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2015; 148(3): 440-449.

48. Katchooi M, Cohanim B, Tai S, et al. Effect of supplemental vibration on orthodontic treatment with aligners: A randomized trial. *Am J Orthod Dentofacial Orthop* 2018; 153(3): 336-346.

49. Miles P, Fisher E. Assessment of the changes in arch perimeter and irregularity in the mandibular arch during initial alignment with the AcceleDent Aura appliance vs no appliance in adolescents: A single-blind randomized clinical trial. *Am J Orthod Dentofacial Orthop* 2016; 150(6): 928-936.

50. Miles P, Smith H, Weyant R, Rinchuse DJ. The effects of a vibrational appliance on tooth movement and patient discomfort: A prospective randomised clinical trial. ***Aust Orthod J*** 2012; 28(2): 213-218.

51. Woodhouse NR, DiBiase AT, Johnson N, et al. Supplemental Vibrational Force During Orthodontic Alignment: A Randomized Trial. ***J Dent Res*** 2015; 94(5): 682-689

52. DiBiase AT, Woodhouse NR, Papageorgiou SN, et al. Effects of supplemental vibrational force on space closure, treatment duration, and occlusal outcome: A multicenter randomized clinical trial. ***Am J Orthod Dentofacial Orthop*** 2018; 153(4): 469-480.

53. Leethanakul C, Suamphan S, Jitpukdeebodintra S, Thongudomporn U, Charoemratrote C. Vibratory stimulation increases interleukin-1 beta secretion during orthodontic tooth movement. ***Angle Orthod*** 2016; 86(1): 74-80.

54. Pavlin D, Anthony R, Raj V, Gakunga PT. Cyclic loading (vibration) accelerates tooth movement in orthodontic patients: A double-blind randomized controlled trial. ***Semin Orthod*** 2015; 21(3): 187-194.

55. Liao Z, Elekdag-Turk S, Turk T, et al. Computational and clinical investigation on the role of mechanical vibration on orthodontic tooth movement. ***J Biomech*** 2017; 60: 57-64.

56. Davidovitch Z, Nicolay OF, Ngan PW, Shanfeld JL. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. ***Dent Clin North Am*** 1988; 32(3): 411-435.

57. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. ***Am J Orthod Dentofacial Orthop*** 2006; 129(4): 1-32.

58. Schwarz AM. Tissue changes incidental to orthodontic tooth movement. ***Int J Orthod, Oral Surg Radio*** 1932; 18(4): 331-352.

59. JN. F. Irregularities of the teeth and their correction. New York: DeVinne Press 1888; 1: 658.

60. Grimm FM. Bone bending, a feature of orthodontic tooth movement. ***Am J Orthod Dentofacial Orthop*** 1972; 62(4): 384-393.

61. Pollack S, Salzstein R, Pienkowski D. The electric double layer in bone and its influence on stress-generated potentials. ***Calcif Tissue Int*** 1984; 36(1): 77-81.

62. Epker BN FH. Correlation of bone resorption and formation with the physical behavior of loaded bone. ***J Dent Res*** 1965; 44: 33-41.

63. Davidovitch Z. Tooth movement. ***Crit Rev Oral Biol Med*** 1991; 2(4): 411-450.

64. Toms SR, Lemons JE, Bartolucci AA, Eberhardt AW. Nonlinear stress-strain behavior of periodontal ligament under orthodontic loading. ***Am J Orthod Dentofacial Orthop*** 2002; 122(2): 174-179.

65. Burstone CJ. The biomechanics of tooth movement. In Kraus BS, Riedel RA (editor): Vistas in Orthodontics, Plliladelphia: Lea & Febiger; 1962: 197-213.

66. Reitan K. Some factors determining the evaluation of forces in orthodontics. ***Am J Orthod Dentofacial Orthop*** 1957; 43(1): 32-45.

67. Storey E SR. Force in orthodontics and its relation to tooth movement. ***Aust Dent J*** 1952; 56: 11-18.

68. Ren Y, Maltha JC, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a systematic literature review. ***Angle Orthod*** 2003; 73(1): 86-92.

69. Insee K, Pothacharoen P, Kongtawelert P, et al. Comparisons of the chondroitin sulphate levels in orthodontically moved canines and the clinical outcomes between two different force magnitudes. ***Eur J Orthod*** 2014; 36(1): 39-46.

70. Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. ***J Dent Res*** 2009; 88(7): 597-608.

71. Chae HS, Park HJ, Hwang HR, et al. The effect of antioxidants on the production of pro-inflammatory cytokines and orthodontic tooth movement. ***Mol Cells*** 2011; 32(2): 189-196.

72. Kindle L, Rothe L, Kriss M, Osdoby P, Collin-Osdoby P. Human microvascular endothelial cell activation by IL-1 and TNF-alpha stimulates the adhesion and transendothelial migration of circulating human CD14+ monocytes that develop with RANKL into functional osteoclasts. ***J Bone Miner Res*** 2006; 21(2): 193-206.

73. Dai J, Rabie AB. VEGF: an essential mediator of both angiogenesis and endochondral ossification. ***J Dent Res*** 2007; 86(10): 937-950.

74. Garlet TP, Coelho U, Silva JS, Garlet GP. Cytokine expression pattern in compression and tension sides of the periodontal ligament during orthodontic tooth movement in humans. ***Eur J Oral Sci*** 2007; 115(5): 355-362.

75. Bletsa A, Berggreen E, Brudvik P. Interleukin-1alpha and tumor necrosis factor-alpha expression during the early phases of orthodontic tooth movement in rats. ***Eur J Oral Sci*** 2006; 114(5): 423-429.

76. Andrade I, Jr., Taddei SR, Garlet GP, et al. CCR5 down-regulates osteoclast function in orthodontic tooth movement. ***J Dent Res*** 2009; 88(11): 1037-1041.

77. Garlet TP, Coelho U, Repeke CE, et al. Differential expression of osteoblast and osteoclast chemmoatractants in compression and tension sides during orthodontic movement. ***Cytokine*** 2008; 42(3): 330-335.

78. Yu X, Huang Y, Collin-Osdoby P, Osdoby P. CCR1 chemokines promote the chemotactic recruitment, RANKL development, and motility of osteoclasts and are induced by inflammatory cytokines in osteoblasts. ***J Bone Miner Res*** 2004; 19(12): 2065-2077.

79. Binder NB, Niederreiter B, Hoffmann O, et al. Estrogen-dependent and C-C chemokine receptor-2-dependent pathways determine osteoclast behavior in osteoporosis. ***Nat Med*** 2009; 15(4): 417-424.

80. Wright LM, Maloney W, Yu X, et al. Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. ***Bone*** 2005; 36(5): 840-853.

81. Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. ***Eur J Orthod*** 2006; 28(3): 221-240.

82. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. ***Arch Biochem Biophys*** 2008; 473(2): 139-146.

83. 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(2): 210-220.

84. Nakao K, Goto T, Gunjigake KK, et al. Intermittent force induces high RANKL expression in human periodontal ligament cells. ***J Dent Res*** 2007; 86(7): 623-628.

85. Park HJ, Baek KH, Lee HL, et al. Hypoxia inducible factor-1alpha directly induces the expression of receptor activator of nuclear factor-kappaB ligand in periodontal ligament fibroblasts. ***Mol Cells*** 2011; 31(6):573-578.

86. Cheung WY, Liu C, Tonelli-Zasarsky RM, Simmons CA, You L. Osteocyte apoptosis is mechanically regulated and induces angiogenesis in vitro. ***J Orthop Res*** 2011; 29(4): 523-530.

87. Yao Z, Xing L, Qin C, Schwarz EM, Boyce BF. Osteoclast precursor interaction with bone matrix induces osteoclast formation directly by an interleukin-1-mediated autocrine mechanism. ***J Biol Chem*** 2008; 283(15): 9917-9924.

88. Kawaguchi H, Chikazu D, Nakamura K, Kumegawa M, Hakeda Y. Direct and indirect actions of fibroblast growth factor 2 on osteoclastic bone resorption in cultures. ***J Bone Miner Res*** 2000; 15(3): 466-473.

89. Masella RS, Meister M. Current concepts in the biology of orthodontic tooth movement. ***Am J Orthod Dentofacial Orthop*** 2006; 129(4): 458-468.

90. Tsubaki M, Kato C, Manno M, et al. Macrophage inflammatory protein-1alpha (MIP-1alpha) enhances a receptor activator of nuclear factor kappaB ligand (RANKL) expression in mouse bone marrow stromal cells and osteoblasts through MAPK and PI3K/Akt pathways. ***Mol Cell Biochem*** 2007; 304(1-2): 53-60.

91. Reijnders CM, Bravenboer N, Tromp AM, Blankenstein MA, Lips P. Effect of mechanical loading on insulin-like growth factor-I gene expression in rat tibia. ***J Endocrinol*** 2007; 192(1): 131-140.

92. Hirukawa K, Miyazawa K, Maeda H, et al. Effect of tensile force on the expression of IGF-I and IGF-I receptor in the organ-cultured rat cranial suture. ***Arch Oral Biol*** 2005; 50(3): 367-372.

93. Roberts WE, Engen DW, Schneider PM, Hohlt WF. Implant-anchored orthodontics for partially edentulous malocclusions in children and adults. ***Am J Orthod Dentofacial Orthop*** 2004; 126(3): 302-304.

94. Suga K, Saitoh M, Fukushima S, et al. Interleukin-11 induces osteoblast differentiation and acts synergistically with bone morphogenetic protein-2 in C3H10T1/2 cells. ***J Interferon Cytokine Res*** 2001; 21(9): 695-707.

95. Hogan BL. Bone morphogenetic proteins in development. ***Curr Opin Genet Dev*** 1996; 6(4): 432-438.

96. Guajardo G, Okamoto Y, Gogen H, et al. Immunohistochemical localization of epidermal growth factor in cat paradental tissues during tooth movement. ***Am J Orthod Dentofacial Orthop*** 2000; 118(2): 210-219.

97. Kohno S, Kaku M, Tsutsui K, et al. Expression of vascular endothelial growth factor and the effects on bone remodeling during experimental tooth movement. ***J Dent Res*** 2003; 82(3): 177-182.

98. Spits H, de Waal Malefyt R. Functional characterization of human IL-10. ***Int Arch Allergy Immunol*** 1992; 99(1): 8-15.

99. Kobayashi Y, Hashimoto F, Miyamoto H, et al. Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor beta and osteoprotegerin expression. ***J Bone Miner Res*** 2000; 15(10): 1924-1934.

100. Govoni KE, Baylink DJ, Mohan S. The multi-functional role of insulin-like growth factor binding proteins in bone. ***Pediatr Nephrol*** 2005; 20(3): 261-268.

101. Han X, Amar S. IGF-1 signaling enhances cell survival in periodontal ligament fibroblasts vs. gingival fibroblasts. ***J Dent Res*** 2003; 82(6): 454-459.

102. Nishijima Y, Yamaguchi M, Kojima T, et al. Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. ***Orthod Craniofac Res*** 2006; 9(2): 63-70.

103. 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(3): 137-142.

104. Dominguez A, Gomez C, Palma JC. Effects of low-level laser therapy on orthodontics: rate of tooth movement, pain, and release of RANKL and OPG in GCF. ***Lasers Med Sci*** 2015; 30(2): 915-923.

105. Grant M, Wilson J, Rock P, Chapple I. Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. ***Eur J Orthod*** 2013; 35(5): 644-651.

106. Kapoor P, Kharbanda OP, Monga N, Miglani R, Kapila S. Effect of orthodontic forces on cytokine and receptor levels in gingival crevicular fluid: a systematic review. ***Prog Orthod*** 2014; 15: 65.

107. Rody WJ, Jr., Wijegunasinghe M, Wiltshire WA, Dufault B. Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. ***Angle Orthod*** 2014; 84(1): 120-126.

108. Toygar HU, Kircelli BH, Bulut S, Sezgin N, Tasdelen B. Osteoprotegerin in gingival crevicular fluid under long-term continuous orthodontic force application. ***Angle Orthod*** 2008; 78(6): 988-993.

109. Barbieri G, Solano P, Alarcon JA, et al. Biochemical markers of bone metabolism in gingival crevicular fluid during early orthodontic tooth movement. ***Angle Orthod*** 2013; 83(1): 63-69.

110. Van Der Velden U. Effect of age on the periodontium. ***J Clin Periodontol*** 1984; 11(5): 281-294.

111. Stahl SS, Tonna EA. H3-proline study of aging periodontal ligament matrix formation: Comparison between matrices adjacent to either cemental or bone surfaces. ***J Periodontal Res*** 1977; 12(4): 318-322.

112. Tuncer BB, Özdemir BÇ, Boynuegri D, et al. OPG-RANKL levels after continuous orthodontic force. ***Gazi Med J*** 2013; 24(2): 33-36.

113. Kim SJ, Park KH, Park YG, Lee SW, Kang YG. Compressive stress induced the up-regulation of M-CSF, RANKL, TNF-alpha expression and the down-regulation of OPG expression in PDL cells via the integrin-FAK pathway. ***Arch Oral Biol*** 2013; 58(6): 707-716.

114. Van Leeuwen EJ, Maltha JC, Kuijpers-Jagtman AM. Tooth movement with light continuous and discontinuous forces in beagle dogs. ***Eur J Oral Sci*** 1999; 107(6): 468-474.

115. Pilon JJ, Kuijpers-Jagtman AM, Maltha JC. Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. ***Am J Orthod Dentofacial Orthop*** 1996; 110(1): 16-23.

116. Köle H. Surgical operations on the alveolar ridge to correct occlusal abnormalities. ***Oral Surg Oral Med Oral Pathol*** 1959; 12(3): 277-288.

117. Generson R, Porter J, Zell A, Stratigos G. Combined surgical and orthodontic management of anterior open bite using corticotomy. ***J Oral Surg*** 1978; 36(3): 216-219.

118. Kerdvongbundit V. Corticotomy--facilitated orthodontics. ***J Dent Assoc Thai*** 1989; 40(6): 284-291.

119. Gantes B, Rathbun E, Anholm M. Effects on the Periodontium Following Corticotomy-Facilitated Orthodontics. Case Reports*. ***J Periodontol*** 1990; 61(4): 234-238.

120. Wilcko WM, Wilcko MT, Bouquot J, Ferguson DJ. Rapid orthodontics with alveolar reshaping: two case reports of decrowding. ***Int J Periodontics Restorative Dent*** 2001; 21(1): 9-20.

121. Nazarov A, Ferguson D, Wilcko W, Wilcko M. Improved orthodontic retention following corticotomy using ABO Objective Grading System. ***J Dent Res*** 2004; 83: 2644.

122. Baloul SS, Gerstenfeld LC, Morgan EF, et al. Mechanism of action and morphologic changes in the alveolar bone in response to selective alveolar decortication–facilitated tooth movement. ***Am J Orthod Dentofacial Orthop*** 2011; 139(4): 83-101.

123. Han X-L, Meng Y, Kang N, Lv T, Bai D. Expression of osteocalcin during surgically assisted rapid orthodontic tooth movement in beagle dogs. ***J Oral Maxillofac Surg*** 2008; 66(12): 2467-2475.

124. Ilizarov G. The Possibilities Offered by Our Method for Lengthening Various Segments in Upper and Lower Limbs. In: Nicoletti B, Kopits S, Ascani E, McKusick V, Dryburgh S, editors. Human Achondroplasia: Springer US; 1988: 323-24.

125. Ren A, Lv T, Kang N, et al. Rapid orthodontic tooth movement aided by alveolar surgery in beagles. ***Am J Orthod Dentofacial Orthop*** 2007; 131(2): 1-60.

126. Sukurica Y, Karaman A, Gürel HG, Dolanmaz D. Rapid canine distalization through segmental alveolar distraction osteogenesis. ***Angle Orthod*** 2007; 77(2): 226-236.

127. Dibart S, Surmenian J, David Sebaoun J, Montesani L. Rapid treatment of Class II malocclusion with piezocision: two case reports. ***Int J Periodontics Restorative Dent*** 2010; 30(5): 487.

128. Mittal S, Sharma R, Singla A. Piezocision assisted orthodontics: a new approach to accelerated orthodontic tooth movement. ***J Innov Dent*** 2011; 1(1): 1-4.

129. Keser EI, Dibart S. Piezocision-assisted Invisalign treatment. ***Compend Contin Educ Dent*** 2011; 32(2): 46-48, 50-51.

130. Maiman TH. Biomedical lasers evolve toward clinical applications. ***Hosp Manage*** 1966; 101(4): 39-41.

131. Lim H-M, Lew KK, Tay DK. A clinical investigation of the efficacy of low level laser therapy in reducing orthodontic postadjustment pain. ***Am J Orthod Dentofacial Orthop*** 1995; 108(6): 614-622.

132. Mester E, Mester AF, Mester A. The biomedical effects of laser application. ***Lasers Surg Med*** 1985; 5(1): 31-39.

133. Tajali SB, MacDermid JC, Houghton P, Grewal R. Effects of low power laser irradiation on bone healing in animals: a meta-analysis. ***J Orthop Surg Res*** 2010;5(1):1.

134. Pires-Oliveira D, Oliveira R, Amadei S, Pacheco-Soares C, Rocha R. Laser 904 nm action on bone repair in rats with osteoporosis. ***Osteoporos Int*** 2010; 21(12): 2109-2114.

135. Kim YD, Kim SS, Hwang DS, et al. Effect of low-level laser treatment after installation of dental titanium implant-immunohistochemical study of RANKL, RANK, OPG: an experimental study in rats. ***Lasers Surg Med*** 2007; 39(5): 441-450.

136. Saito S, Shimizu N. Stimulatory effects of low-power laser irradiation on bone regeneration in midpalatal suture during expansion in the rat. ***Am J Orthod Dentofacial Orthop*** 1997; 111(5): 525-532.

137. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. ***Lasers Surg Med*** 2000; 26(3): 282-291.

138. Fujita S, Yamaguchi M, Utsunomiya T, Yamamoto H, Kasai K. Low-energy laser stimulates tooth movement velocity via expression of RANK and RANKL. ***Orthod Craniofac Res*** 2008;11(3): 143-155.

139. 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(1): 38-43.

140. Davidovitch Z, Finkelson MD, Steigman S, et al. 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 Dentofacial Orthop*** 1980; 77(1):33-47.

141. Kakehi N, Yamazaki T, Tsugawa W, Sode K. A novel wireless glucose sensor employing direct electron transfer principle based enzyme fuel cell. ***Biosens Bioelectron*** 2007; 22(9): 2250-2255.

142. Kolahi J, Abrishami M, Davidovitch Z. Microfabricated biocatalytic fuel cells: A new approach to accelerating the orthodontic tooth movement. ***Med hypotheses*** 2009; 73(3): 340-41.

143. Zhang C, Li J, Zhang L, et al. Effects of mechanical vibration on proliferation and osteogenic differentiation of human periodontal ligament stem cells. ***Arch Oral biol*** 2012; 57(10): 1395-1407.

144. Xue H, Zheng J, Cui Z, et al. Low-intensity pulsed ultrasound accelerates tooth movement via activation of the BMP-2 signaling pathway. ***PLoS One*** 2013; 8(7): e68926.

145. Mansfield NJ. Human response to vibration. Boca Raton, Florida; ***CRC Press*** 2005:1-26.

146. Mudd LM, Fornetti W, Pivarnik JM. Bone mineral density in collegiate female athletes: comparisons among sports. ***J Athl Train*** 2007; 42(3): 403.

147. Suominen H. Bone mineral density and long term exercise. ***Sports Med*** 1993; 16(5): 316-330.

148. Keller TS, Strauss AM, Szpalski M. Prevention of bone loss and muscle atrophy during manned space flight. ***Microgravity Q*** 1992;2(2):89-102.

149. Hert J, Liskova M, Landa J. Reaction of bone to mechanical stimuli. 1. Continuous and intermittent loading of tibia in rabbit. ***Folia Morphol (Praha)*** 1971;19(3):290-300.

150. Liskova M, Hert J. Reaction of bone to mechanical stimuli. 2. Periosteal and endosteal reaction of tibial diaphysis in rabbit to intermittent loading. ***Folia Morphol (Praha)*** 1971; 19(3): 301-317.

151. Lanyon LE, Smith RN. Bone strain in the tibia during normal quadrupedal locomotion. ***Acta Orthop Scand 1970***; 41(3): 238-48.

152. Lanyon LE, Rubin C. Static vs dynamic loads as an influence on bone remodelling. ***J Biomech*** 1984; 17(12): 897-905.

153. Rubin C, Gross T, Qin YX, et al. Differentiation of the bone-tissue remodeling response to axial and torsional loading in the turkey ulna. ***J Bone Joint Surg Am*** 1996; 78(10): 1523-1533.

154. Rubin CT, Lanyon LE. Regulation of bone formation by applied dynamic loads. ***J Bone Joint Surg Am*** 1984; 66(3): 397-402.

155. Rubin CT, Lanyon LE. Regulation of bone mass by mechanical strain magnitude. ***Calcif Tissue Int*** 1985; 37(4): 411-7.

156. Oxlund B, Ortoft G, Andreassen TT, Oxlund H. Low-intensity, high-frequency vibration appears to prevent the decrease in strength of the femur and tibia associated with ovariectomy of adult rats. ***Bone*** 2003; 32(1): 69-77.

157. Verschueren SM, Roelants M, Delecluse C, et al. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in

postmenopausal women: a randomized controlled pilot study. ***J Bone Miner Res*** 2004; 19(3): 352-359.

158. Gusi N, Raimundo A, Leal A. Low-frequency vibratory exercise reduces the risk of bone fracture more than walking: a randomized controlled trial. ***BMC Musculoskelet Disord*** 2006; 7: 92.
159. Christiansen BA, Silva MJ. The effect of varying magnitudes of whole-body vibration on several skeletal sites in mice. ***Ann Biomed Eng*** 2006; 34(7): 1149-1156.
160. Xie L, Jacobson JM, Choi ES, et al. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. ***Bone*** 2006; 39(5): 1059-1066.
161. Xie L, Rubin C, Judex S. Enhancement of the adolescent murine musculoskeletal system using low-level mechanical vibrations. ***J Appl Physiol*** 2008; 104(4): 1056-1062.
162. Iwamoto J, Takeda T, Sato Y, Uzawa M. Effect of whole-body vibration exercise on lumbar bone mineral density, bone turnover, and chronic back pain in post-menopausal osteoporotic women treated with alendronate. ***Aging Clin Exp Res*** 2005; 17(2): 157-163.
163. Cowin SC. Mechanosensation and fluid transport in living bone. ***J Musculoskelet Neuronal Interact*** 2002; 2(3): 256-260.
164. Burger EH, Klein-Nulend J. Mechanotransduction in bone—role of the lacuno-canalicular network. ***FASEB J*** 1999; 13(9001): 101-112.
165. McAllister TN, Du T, Frangos JA. Fluid shear stress stimulates prostaglandin and nitric oxide release in bone marrow-derived preosteoclast-like cells. ***Biochem Biophys Res Commun*** 2000; 270(2): 643-648.
166. Tan SD, de Vries TJ, Kuijpers-Jagtman AM, et al. Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption. ***Bone*** 2007; 41(5): 745-751.
167. Roberts WE, Huja S, Roberts JA. Bone modeling: biomechanics, molecular mechanisms, and clinical perspectives. ***Semin Orthod*** 2004; 10(2): 123-161.
168. Graber LW, Vanarsdall Jr RL, Vig KW. Orthodontics: current principles and techniques. St. Loius, Missouri, USA: Elsevier Health Sciences; 2011: 259-263
169. Henao SP, Kusy RP. Evaluation of the frictional resistance of conventional and self-ligating bracket designs using standardized archwires and dental typodonts. ***Angle Orthod*** 2004; 74(2): 202-211.

170. Bartzela T, Türp JC, Motschall E, Maltha JC. Medication effects on the rate of orthodontic tooth movement: a systematic literature review. ***Am J Orthod Dentofacial Orthop*** 2009; 135(1): 16-26.

171. Rubin C, Recker R, Cullen D, et al. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: a clinical trial assessing compliance, efficacy, and safety. ***J Bone Miner Res*** 2004; 19(3): 343-351.

172. Kau CH. A radiographic analysis of tooth morphology following the use of a novel cyclical force device in orthodontics. ***Head Face Med*** 2011; 7: 1-5.

173. Andrade I, Taddei SR, Souza PE. Inflammation and tooth movement: the role of cytokines, chemokines, and growth factors. ***Semin Orthod*** 2012;18: 257-269.

174. Olson JE, Liu Y, Nickel JC, Walker MP, Iwasaki LR. Archwire vibration and stick-slip behavior at the bracket-archwire interface. ***Am J Orthod Dentofacial Orthop*** 2012; 142(3): 314-322.

175. Seo YJ, Lim BS, Park YG, et al. Effect of tooth displacement and vibration on frictional force and stick-slip phenomenon in conventional brackets: a preliminary in vitro mechanical analysis. ***Eur J Orthod*** 2015; 37(2): 158-163.

176. Seo YJ, Lim BS, Park YG, et al. Effect of self-ligating bracket type and vibration on frictional force and stick-slip phenomenon in diverse tooth displacement conditions: an in vitro mechanical analysis. ***Eur J Orthod*** 2015; 37(5): 474-480.

177. Kau CH, Nguyen JT, English J. The clinical evaluation of a novel cyclical force generating device in orthodontics. ***Orthod Pract*** 2010; 1(1): 1-4.

178. Kalajzic Z, Peluso EB, Utreja A, et al. Effect of cyclical forces on the periodontal ligament and alveolar bone remodeling during orthodontic tooth movement. ***Angle Orthod*** 2014; 84(2): 297-303.

179. Woodhouse NR, DiBiase AT, Johnson N, et al. Supplemental vibrational force during orthodontic alignment: a randomized trial. ***J Dent Res*** 2015; 94(5): 682-689.

180. Miles P, Fisher E, Pandis N. Assessment of the rate of premolar extraction space closure in the maxillary arch with the AcceleDent Aura appliance vs no appliance in adolescents: A single-blind randomized clinical trial. ***Am J Orthod Dentofacial Orthop*** 2018; 153(1): 8-14.

181. Anderson DM, Maraskovsky E, Billingsley WL, et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. ***Nature*** 1997; 390(6656): 175-179.

182. Suzuki J, Ikeda T, Kuroyama H, et al. Regulation of osteoclastogenesis by three human RANKL isoforms expressed in NIH3T3 cells. ***Biochem Biophys Res Commun*** 2004; 314(4): 1021-1027.

183. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. ***Cell*** 1998; 93(2): 165-176.

184. Nagai M, Kyakumoto S, Sato N. Cancer cells responsible for humoral hypercalcemia express mRNA encoding a secreted form of ODF/TRANCE that induces osteoclast formation. ***Biochem Biophys Res Commun*** 2000; 269(2): 532-536.

185. Feng X. RANKing intracellular signaling in osteoclasts. ***IUBMB Life*** 2005; 57(6): 389-395.

186. Blair JM, Zhou H, Seibel MJ, Dunstan CR. Mechanisms of disease: roles of OPG, RANKL and RANK in the pathophysiology of skeletal metastasis. ***Nat Clin Pract Oncol*** 2006; 3(1): 41-49.

187. Whyte MP, Obrecht SE, Finnegan PM, et al. Osteoprotegerin deficiency and juvenile Paget's disease. ***N Engl J Med*** 2002; 347(3): 175-184.

188. Hughes AE, Ralston SH, Marken J, et al. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. ***Nat Genet*** 2000; 24(1): 45-48.

189. Pichler K, Loreto C, Leonardi R, et al. RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training) in rat with glucocorticoid-induced osteoporosis. ***Histol Histopathol*** 2013; 28(9): 1185-1196.

190. Bergstrom I, Parini P, Gustafsson SA, Andersson G, Brinck J. Physical training increases osteoprotegerin in postmenopausal women. ***J Bone Miner Metab*** 2012; 30(2): 202-207.

191. Chung SL, Leung KS, Cheung WH. Low-magnitude high-frequency vibration enhances gene expression related to callus formation, mineralization and remodeling during osteoporotic fracture healing in rats. ***J Orthop Res*** 2014; 32(12): 1572-1579.

192. Hou WW, Zhu ZL, Zhou Y, Zhang CX, Yu HY. Involvement of Wnt activation in the micromechanical vibration-enhanced osteogenic response of osteoblasts. ***J Orthop Sci*** 2011; 16(5): 598-605.

193. Nakano Y, Yamaguchi M, Fujita S, et al. Expressions of RANKL/RANK and M-CSF/c-fms in root resorption lacunae in rat molar by heavy orthodontic force. ***Eur J Orthod*** 2011; 33(4): 335-343.

194. Lau E, Al-Dujaili S, Guenther A, et al. Effect of low-magnitude, high-frequency vibration on osteocytes in the regulation of osteoclasts. ***Bone*** 2010; 46(6): 1508-1515.

195. Wu SH, Zhong ZM, Chen JT. Low-magnitude high-frequency vibration inhibits RANKL-induced osteoclast differentiation of RAW264.7 cells. ***Int J Med Sci*** 2012; 9(9): 801-807.

196. Dixon V, Read MJF, O'Brien KD, Worthington HV, Mandall NA. A randomized clinical trial to compare three methods of orthodontic space closure. ***J Orthod*** 2002; 29(1): 31-36.

197. Welham SJ, Gezan SA, Clark S, Mead A. Statistical methods in biology: Design and analysis of experiments and regression. Abingdon, UK: Taylor & Francis; 2014: 48.

198. Lima IFP, de Andrade Vieira W, de Macedo Bernardino I, et al. Influence of reminder therapy for controlling bacterial plaque in patients undergoing orthodontic treatment: A systematic review and meta-analysis. ***Angle Orthod*** 2018; 88(4): 483-493.

199. Roter DL, Hall JA, Merisca R, et al. Effectiveness of Interventions to Improve Patient Compliance: A Meta-Analysis. ***Med Care*** 1998; 36(8): 1138-1161.

200. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. ***J Periodontal Res*** 1986; 21(2): 101-112.

201. Jang I, Tanaka M, Koga Y, et al. A novel method for the assessment of three-dimensional tooth movement during orthodontic treatment. ***Angle Orthod*** 2009; 79(3): 447-453.

202. Dahlberg G. Statistical Methods for Medical and Biological Students. ***Br Med J*** 1940; 2: 358-359.

203. Nakashima T, Kobayashi Y, Yamasaki S, et al. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. ***Biochem Biophys Res Commun*** 2000; 275(3): 768-775.

204. Kaneko S, Ohashi K, Soma K, Yanagishita M. Occlusal hypofunction causes changes of proteoglycan content in the rat periodontal ligament. ***J Periodontal Res*** 2001; 36(1): 9-17.

205. Muramoto T, Takano Y, Soma K. Time-related changes in periodontal mechanoreceptors in rat molars after the loss of occlusal stimuli. ***Arch Histol Cytol*** 2000; 63(4): 369-380.

206. Enokida M, Kaneko S, Yanagishita M, Soma K. Influence of Occlusal Stimuli on the Remodelling of Alveolar Bone in a Rat Hypofunction-Recovery Model. ***J Oral Biosci*** 2005; 47(4): 321-334.

207. Frost HM. Skeletal structural adaptations to mechanical usage (SATMU): 2. Redefining Wolff's law: the remodeling problem. ***Anat Rec*** 1990; 226(4): 414-422.

208. Frost HM. Skeletal structural adaptations to mechanical usage (SATMU): 1. Redefining Wolff's law: the bone modeling problem. ***Anat Rec*** 1990; 226(4): 403-413.

209. Reynolds JJ, Meikle MC. Mechanisms of connective tissue matrix destruction in periodontitis. ***Periodontol 2000*** 1997; 14: 144-157.

210. Redlich K, Hayer S, Ricci R, et al. Osteoclasts are essential for TNF-alpha-mediated joint destruction. ***J Clin Invest*** 2002; 110(10): 1419-1427.

211. Ericsson I, Thilander B, Lindhe J, Okamoto H. The effect of orthodontic tilting movements on the periodontal tissues of infected and non-infected dentitions in dogs. ***J Clin Periodontol*** 1977; 4(4): 278-293.

212. Artun J, Urbye KS. The effect of orthodontic treatment on periodontal bone support in patients with advanced loss of marginal periodontium. ***Am J Orthod Dentofacial Orthop*** 1988; 93(2): 143-148.

213. Heritier SR, Gebski VJ, Keech AC. Inclusion of patients in clinical trial analysis: the intention-to-treat principle. ***Med J Aust*** 2003; 179(8): 438-440.

214. Wertz RT. Intention to treat: Once randomized, always analyzed. ***Clin Aphasiol*** 1995; 23: 57-64.

APPENDICES

APPENDICES

ที่ ศธ 0521.1.03/806

คณะทันตแพทยศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ไปรษณีย์เลขที่ 17
ที่ทำการไปรษณีย์โทรเลขคอหงส์
อ.หาดใหญ่ จ.สงขลา 90112

หนังสือฉบับนี้ให้ไว้เพื่อรับรองว่า

โครงการวิจัยเรื่อง "การศึกษาเปรียบเทียบการกระตุ้นการเคลื่อนฟันทางทันตกรรมจัดฟันด้วยการสั่นที่ความถี่ต่างๆ ต่ออัตราส่วนการหลั่งของแรงค์แอลต่อโอพีจี"

รหัสโครงการ EC5805-21-P- HR

หัวหน้าโครงการ ทันตแพทย์ณัฐชนน ศิริพันธ์

สังกัดหน่วยงาน นักศึกษาหลังปริญญา ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์

ได้ผ่านการพิจารณาและได้รับความเห็นชอบจากคณะกรรมการจริยธรรมในการวิจัย (Research Ethics Committee) ซึ่งเป็นคณะกรรมการพิจารณาศึกษาการวิจัยในคนของคณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ดำเนินการให้การรับรองโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นสากล ได้แก่ Declaration of Helsinki, the Belmont Report, CIOMS Guidelines และ the International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

ในคราวประชุมครั้งที่ 5/2558 **เมื่อวันที่** 4 มิถุนายน 2558

ให้ไว้ ณ วันที่ 7 ก.ค. 2558

(ผู้ช่วยศาสตราจารย์ ดร.ทพญ.ศรีสุรางค์ สุทธปรียาศรี)
ประธานคณะกรรมการจริยธรรมในการวิจัย

กรรมการ (ผู้ช่วยศาสตราจารย์ ทพ.นพ.สุรพงษ์ วงศ์วัชรานนท์)

กรรมการ (ผู้ช่วยศาสตราจารย์ ดร. ทพญ.สุพัชรินทร์ พิวัฒน์)

กรรมการ (รองศาสตราจารย์ นพ.พรชัย สถิรปัญญา)

กรรมการ (อาจารย์ ทพ.กมลพันธ์ เนื่องศรี)

กรรมการ (ผู้ช่วยศาสตราจารย์ ดร.ทพญ.อังคณา เธียรมนตรี)

กรรมการ (อาจารย์วศิน สุวรรณรัตน์)

กรรมการ (ผู้ช่วยศาสตราจารย์ ดร.ทพญ.สุวรรณา จิตภักดีบดินทร์)

กรรมการ (นายบุญสิทธิ์ บัวบาน)

RESEARCH ETHICS COMMITTEE (REC)
BUILDING 1 5TH FLOOR ROOM 504
TEL. 66-74-287533, 66-74-287504
FAX. 66-74-287533

FACULTY OF DENTISTRY
PRINCE OF SONGKLA UNIVERSITY
HADYAI, SONGKHLA 90112, THAILAND
TEL. 66-74-212914, 66-74-429871, 66-74-287500
FAX. 66-74-429871, 66-74-212922

Documentary Proof of Ethical Clearance

Research Ethics Committee (REC)

Faculty of Dentistry, Prince of Songkla University

The Project Entitled A Comparative Study of Different Vibratory Stimuli Frequencies During Orthodontic Tooth Movement on the Secretion of RANKL : OPG Ratio

REC Project No. : EC5805-21-P-HR

Principal Investigator : Mr. Natchanon Siriphan

Approved by Research Ethics Committee (REC), Faculty of Dentistry, Prince of Songkla University.

This is to certify that REC is in full Compliance with International Guidelines for Human Research Protection such as the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Date of Approval : 7 JULY 2015 **No. of Approval** : MOE 0521.1.03/ 806

(Asst. Prof. Dr. Srisurang Suttapreyasri)
Chairman of Research Ethics Committee

Surapong Vongvatchranon
(Asst. Prof.Surapong Vongvatchranon)

Supatcharin Piwat
(Asst. Prof. Dr. Supatcharin Piwat)

(Assoc. Prof. Pornchai Sathirapanya)

(Mr. Kamolphan Nuangsri)

(Asst. Prof. Dr. Angkana Thearmontree)

(Mr. Wasin Suwannarat)

(Asst. Prof. Dr. Suwanna Jitpakdeebodintra)

(Mr. Boonsit Buaban)

VITAE

Name Mr. Natchanon Siriphan

Student ID 5610830004

Education Attainment

Degree	Name of Institution	Year of Graduation
Doctor of Dental Surgery	Prince of Songkla University	2552

Scholarships and Awards during Enrolment

Graduate School Research Scholarship, Prince of Songkla University 2013-2016

Faculty of Dentistry Scholarship, Prince of Songkla University 2013-2016

Work-Position and Address

Dentist, Panarae hospital, Tumbon Thakam, Panarae, Pattani, 94130

E-mail: tukmo_ns@hotmail.com

List of Publication and Proceeding

Siriphan N, Thongudomporn U. The effect of two different vibratory frequencies on orthodontic tooth movement rate. Proceedings of the 2017 American Association of Orthodontists (AAO) Annual Conference; 2017 Apr 21-25; San Diego, California, USA.

Siriphan N, Thongudomporn U. The effect of vibratory stimuli on the rate of maxillary canine distalization: a pilot study. Proceeding of the 15th National Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT2017); 2017 Jul 19-21; Phitsanulok, Thailand.

N.Siriphan, C.Leethanakul, U.Thongudomporn. Effects of two frequencies of vibration on the maxillary canine distalization rate and RANKL and OPG secretion: a randomized controlled trial. ***Orthod Craniofac Res***; 22(2): (Accepted, first published 13 February 2019)