THE EFFECT OF OPTIMUM MICRO-OSTEOPERFORATION ACCELERATED TOOTH MOVEMENT ON MANDIBULAR TRABECULAE BONE VOLUME FRACTION – A RANDOMIZED CONTROLLED TRIAL

TEH NG HENG KHIANG

FACULTY OF DENTISTRY
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

THE EFFECT OF OPTIMUM MICRO-OSTEOPERFORATION ACCELERATED TOOTH MOVEMENT ON MANDIBULAR TRABECULAE BONE VOLUME FRACTION – A RANDOMIZED CONTROLLED TRIAL

TEH NG HENG KHIANG

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF ORTHODONTICS

FACULTY OF DENTISTRY
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: TEH NG HENG KHIANG

Matric No: DGD150001

Name of Degree: Masters of Orthodontics

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

The Effect of Optimum Micro-Osteoperforation Accelerated Tooth Movement On Mandibular Trabeculae Bone Volume Fraction – A Randomized Controlled Trial

Field of Study: Orthodontics

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature: Date: 21/5/2019

Subscribed and solemnly declared before,

Witness's Signature: Date: 21/5/2019

Name:

Designation:

[THE EFFECT OF OPTIMUM MICRO-OSTEOPERFORATION ACCELERATED TOOTH MOVEMENT ON MANDIBULAR TRABECULAE BONE VOLUME FRACTION – A RANDOMIZED CONTROLLED TRIAL]

ABSTRACT

Objective: To investigate the effect of Micro-osteoperforation (MOP) on the horizontal and vertical distribution of mandibular trabeculae pattern in relation to different MOP intervals on canine retraction. **Method:** This was a split-mouth randomized clinical trial of thirty participants equally randomized into three groups of different MOP intervals (four, eight and twelve weekly). Cone Beam Computed Tomography (CBCT) images (70kV, 10mA, 10.80 seconds, field of view (FOV) of 5cm x 5cm and voxel size of 76µm) were taken to assess the bone volume fraction (BV/TV) in horizontal and vertical planes. **Results:** BV/TV was significantly reduced (mean difference 9.79%, SD 11.89; 95% CI of 4.77 - 14.81; p = 0.00) and canine retraction significantly increased (mean difference -1.25mm/4months, SD 0.79; 95% CI -1.59 - -0.92; p = 0.00) at MOP sites as compared to control sites. MOP significantly changed the vertical and horizontal patterns of trabeculae bone with lower BV/TV in the areas nearest to intervention, as compared to the control sites. Only the 4 weekly MOP interval group showed significant decrease in BV/TV (mean difference 14.73% SD 12.88; 95%; CI 3.96 – 25.50; p = 0.01) despite significant increase in canine retraction rate for all interval groups. With the use of MOP, BV/TV was found to be inversely correlated to the rate of canine retraction (r = -0.425; p = 0.039). **Conclusions:** There is a moderate inverse relationship of a significant reduction of bone volume fraction as canine retraction rate significantly increased. The bone volume fraction reduction was found significant for the four weekly MOP interval, and has not been proven to extend beyond the interdental region of the intervention.

Keywords: accelerated tooth movement; micro-osteoperforation; bone volume fraction; BV/TV

[KESAN OPTIMUM MIKRO-OSTEOPERFORASI PADA PECAHAN ISIPADU TULANG RAHANG BAWAH UNTUK MEMPERCEPATKAN KADAR PERGERAKAN ORTODONTIK GIGI – PERCUBAAN KLINIKAL TERKAWAL SECARA RAWAK]

ABSTRAK

Objektif: Kajian ini bertujuan untuk menyelidik kesan selang masa berbeza mikro-osteoperforasi (MOP) pada corak mendatar dan menegak trabeculae tulang rahang bawah untuk mempercepat kadar pergerakan ortodontik gigi taring. **Kaedah:** Kajian ini merupakan kajian 'split mouth' terkawal secara rawak yang melibatkan tiga puluh peserta yang dibahagikan secara rawak kepada 3 kumpulan berlainan berdasarkan selang masa MOP empat, lapan dan dua belas minggu. Corak mendatar dan menegak pecahan isipadu tulang (BV/TV) disiasat dengan menggunakan pengimejan 'Cone Beam Computed Tomography' (CBCT) (70kV, 10mA, 10.80 saat, 'field of view' (FOV) of 5cm x 5cm and 'voxel size' of 76µm). **Keputusan:** Di kawasan MOP, BV/TV berkurang (perbezaan min 9.79%, SD 11.89; 95% CI of 4.77 - 14.81; p = 0.00) dan kadar pergerakan gigi taring bertambah (perbezaan min -1.25mm/4 bulan, SD 0.79; 95% CI -1.59 - -0.92; p = 0.00) secara ketara. MOP ketara mengubah corak mendatar dan menegak trabeculae tulang, dengan pengurangan ketara di kawasan berdekatan dengan MOP. Hanya MOP selang masa 4 minggu sahaja yang menunjukkan pengurangan ketara BV/TV (perbezaan min 14.73% SD 12.88; 95%; CI 3.96 – 25.50; p = 0.01), walaupun kadar pergerakan gigi taring ketara bertambah untuk semua kumpulan MOP. MOP juga menunjukkan hubungan korelasi songsang antara BV/TV dengan kadar pergerakan gigi taring (r = -0.425; p = 0.039). **Kesimpulan:** Pengurangan signifikan pecahan isipadu tulang didapati berhubungan songsang secara sederhana dengan penambahan signifikan kadar pergerakan gigi taring. Pengurangan pecahan isipadu tulang didapati signifikan untuk

selang masa 4 minggu dan tidak terbukti berkesan di luar kawasan antara akar tempat MOP.

Kata kunci: mempercepatkan kadar pergerakan ortodontik gigi; mikro-osteoperforasi; pecahan isipadu tulang; BV/TV

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my supervisor, Associate Professor Dr. Wey Mang Chek and my co-supervisor, Associate Professor Dr. Norliza Ibrahim for their constant guidance and advice. Thank you for never giving up on me. I am also very grateful to Dr. Saritha Sivarajan for conducting the clinical part of the trial and for all the help, advice and support you have given me throughout this research project; and also to Dr. Muhammad Khan Asif for his assistance in statistical analysis and CBCT data analysis. Special thank you for the technicians in the Radiology Department who assisted me with the radiographic data. I would like to acknowledge Faculty of Dentistry, University of Malaya for the financial assistance granted for this research. This research was supported by Postgraduate Research Fund by Coursework (PPPC) Faculty of Dentistry, University of Malaya (PPPC/C1-2016/DGD/02). Finally, I am truly blessed with the never-ending support from my family and my colleagues. Thank you very much. None of this would be possible without you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

3D	:	Three-dimensional
ANOVA	:	One-way Analysis of variance
BMP	:	Bitmap image file type
BV/TV	:	Bone volume over total volume
CBCT	:	Cone beam computed tomography
CEJ	:	Cemento-enamel junction
CI	:	Confidence interval
CNR	:	Contrast-to-noise ratio
CT	:	Computed tomography
DICOM®	:	Digital Imaging and Communications in Medicine file type
FOV	:	Field of view
H1	:	Horizontal section 1
H2	:	Horizontal section 2
H3	:	Horizontal section 3
HU	:	Hounsfield units
ICC	:	Intra class correlation coefficient
IL-1	:	Interleukin 1
IL-6	:	Interleukin 6
MBT	:	McLaughlin, Bennett, Travisi
MOP	:	Micro-osteoperforation
MOPs	:	Micro-osteoperforations
NiTi	:	Nickel Titanium
OPG	:	Osteoprotegerin
PDL	:	Periodontal ligament

PGE2	:	Prostaglandin E2
RAP	:	Regional accelerated phenomenon
RANK	:	Receptor activator of nuclear factor-kappaB
RANKL	:	Receptor activator of nuclear factor-kappaB ligand
RCT	:	Randomized controlled trial
ROI	:	Region of interest
SD	:	Standard deviation
SS	:	Stainless steel
TAD	:	Temporary anchorage device
V1	:	Vertical section 1
V2	:	Vertical section 2
V3	:	Vertical section 3

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Orthodontists have always been intrigued with methods to hasten orthodontic tooth movement and shorten orthodontic treatment duration, as the average orthodontic treatment duration takes about 20.02 months (95% confidence interval, 19.71, 20.32 months) and consist of around 17.81 treatment visits (95% confidence interval, 15.47, 20.15 visits) (Tsichlaki, Chin, Pandis, & Fleming, 2016). Prolonged treatment duration consumes clinician and patient's time and resources, and is a risk factor associated with the adverse effects of orthodontic treatment (Travess, Roberts-Harry, & Sandy, 2004; Wishney, 2017). Therefore it is in the best interest of both patient and orthodontist that treatment duration be as short as possible.

Treatment duration is influenced by multiple factors such as severity of initial malocclusion, orthodontic technique employed, patient compliance, clinician skill (Mavreas & Athanasiou, 2008) and of course, rate of orthodontic tooth movement (Nimeri, Kau, Abou-Kheir, & Corona, 2013).

The rate of orthodontic tooth movement is controlled by alveolar bone modelling and remodelling and its associated cells and mediators (Burstone, 1962; Krishnan & Davidovitch, 2006, 2009). These bone cells consist of oseteoblasts (mainly in charge of bone formation), osteoclasts (mainly in charge of bone resorption) and osteocytes which are actively involved in bone resorption-formation cycles. The activity of these cells are regulated by both biochemical and mechanical factors (Huang, Williams, & Kyrkanides, 2014). Therefore any alteration to the biology of the bone modelling and remodelling will induce changes in the rate of orthodontic tooth movement (H. Huang et al., 2014; Verna, Dalstra, & Melsen, 2000).

There are many ways to accelerate the rate of orthodontic tooth movement (Nimeri et al., 2013). The many methods can be divided into surgical and non-surgical interventions. Examples of non-surgical adjunctive interventions include resonance vibration, pulsed electromagnetic fields, low-intensity laser irradiation, electric currents and pharmacological approaches (El-Angbawi, McIntyre, Fleming, & Bearn, 2015). Surgical approaches to accelerate orthodontic tooth movement rate consist of corticortomies, piezocisions and micro-osteoperforation (MOP) (Fleming, Fedorowicz, Johal, El-Angbawi, & Pandis, 2015).

Micro-osteoperoration (MOP) is one such method that has successfully accelerated orthodontic tooth movement (M. Alikhani et al., 2013; Sivarajan, Doss, Papageorgiou, Cobourne, & Wey, 2018; Teixeira et al., 2010). MOP induces the regional accelerated phenomenon (RAP) which is the basis for accelerated orthodontics (Tsai, Yang, Hsieh, & Yang, 2016; Verna, 2016; Verna, Zaffe, & Siciliani, 1999; Wilcko, Wilcko, Bouquot, & Ferguson, 2001). The increased rates of bone remodelling and catabolism activities associated with RAP can be observed as trabecular alveolar bone volume fraction (BV/TV) reduction (Baloul et al., 2011; Tsai et al., 2016), and measured via Cone Beam Computed Tomography (CBCT) images (Ibrahim et al., 2014).

1.1 Importance of Proposed Research

MOP has been reported to increase rate of orthodontic tooth movement by two times and shorten treatment duration by 60% (M. Alikhani et al., 2013). Animal studies have shown that there is alveolar bone volumetric fraction (BV/TV) reduction associated with MOP interventions (Sugimori et al., 2018; Tsai et al., 2016). However no human studies have reported on this association. Better understanding of the spread of mandibular trabeculae pattern changes following an effective MOP interval would enable the

clinician to actualize this procedure clinically. By determining the spread of MOP effect related to MOP application interval, this would serve as a clinical guide to ascertain the frequency and sites of MOP that can best lead to shorter orthodontic treatment duration.

1.2 Aims of Study

To investigate the effect of micro-osteoperforation (MOP) on the horizontal and vertical distribution of mandibular trabeculae pattern in relation to different MOP intervals on canine retraction.

1.3 Objectives

i. To compare the vertical and horizontal distribution of mandibular trabeculae pattern between MOP intervention and control side.
ii. To investigate the distribution of mandibular trabeculae pattern between different intervention intervals
iii. To correlate the vertical and horizontal distribution of mandibular trabeculae pattern in relation to rate of canine tooth movement.

1.4 Null Hypothesis

i. There is no statistically significant difference between the vertical and horizontal distribution of mandibular trabeculae pattern between MOP intervention and control side.
ii. There is no statistically significant difference between the distribution of mandibular trabeculae pattern of different intervention intervals.

iii. There is no statistically significant relationship between the vertical and horizontal distribution of mandibular trabeculae pattern in relation to the rate of canine tooth movement.

CHAPTER 2: LITERATURE REVIEW

Orthodontics is the science to correct the malocclusion of teeth (Angle, 1907). Throughout history, various methods employed to straighten teeth generally involve methods to exert force resulting in teeth moving into alignment. Just like physiological forces from lip and tongue, or habits such as thumb sucking (Ackerman & Proffit, 1997; Proffit, 1978), orthodontic wires can exert pressure needed to move teeth, subsequent to bone resorption on the pressure side and bone apposition on the tension side (Reitan, 1967; Rygh, 1974; Schwarz, 1932). Bone resorption and apposition is bone remodelling which is part of bone homeostasis (Ralston, 2017).

2.1 Stages of Orthodontic Tooth Movement

Orthodontic tooth movement can be divided into 3 phases (Burstone, 1962), an initial phase where immediate tooth movement occurs due to displacement of tooth in its socket, followed by a lag phase with very little to no tooth movement, finally a tooth movement phase where the rate of tooth movement increases. These tooth movement patterns have been confirmed in animal models which mimic the tooth movement phases in humans (Pilon, Kuijpers-Jagtman, & Maltha, 1996; Ren, Maltha, & Kuijpers-Jagtman, 2003). The lag phase is due to development of hyalinised areas at the PDL which inhibits orthodontic tooth movement. Only with removal of the necrotic debris can bone remodelling take place and the subsequent orthodontic tooth movement resume (Krishnan & Davidovitch, 2006; Rygh, 1974). Forces that exceed capillary blood pressure of 20-25 g/cm^2 of root surface will cause hyalinisation of PDL to occur (Burstone, 1962). Unfortunately, it is impossible to accurately place that exact amount of pressure to move teeth orthodontically, hence the inevitable development of hyalinised areas which causes the lag phase. Therefore it is apparent that the determining factor of rate of orthodontic tooth

movement is the degree of PDL hyalinization and the rate of its removal (Roberts, Huja, & Roberts, 2004; von Bohl & Kuijpers-Jagtman, 2009).

2.2 Regional Acceleratory Phenomenon

Regional acceleratory phenomenon (RAP) is a ubiquitous post-injury tissue reaction to noxious stimuli (H. M. Frost, 1983). There is increase of bone remodelling units and activity characterised by acceleration of local bone turnover. The RAP is a necessary step for healing as it increases the rate of bone remodelling especially after injury (Harold M. Frost, 1994). Noxious stimuli such as tooth extractions, bone fractures, implant placement, periodontal surgery and orthodontic tooth movement can trigger RAP. A transient burst of localised remodelling was observed following mucoperiosteal surgery in an animal model (Yaffe, Fine, & Binderman, 1994). Noxious stimuli by selective alveolar decortication also triggered increased turnover of alveolar bone, increased osteoclasts and catabolic rate, increased osteoblasts and anabolic rate and produce transiently less calcified bone with an increased rate of orthodontic tooth movement (Sebaoun et al., 2008). This increased rate of resorption-formation cycle or bone remodelling resembles RAP and is the reason behind accelerated orthodontics (Wilcko et al., 2001).

Bone remodelling is a coupled process of activation, resorption, reversal and formation cycles which replaces damaged bone with new bone (Hadjidakis Dimitrios & Androulakis Ioannis, 2007). The process is performed by osteoclasts and osteoblast and is modulated by a host of biochemical and mechanical factors (H. Huang et al., 2014). Noxious stimuli such as alveolar decortication and micro-osteoperforation (MOP) have been shown to increase the number and function of the osteoclasts and osteoblasts and intensify resorption formation cycles (Shih & Norrdin, 1985; Tsai et al., 2016; Verna et

al., 2000; Verna et al., 1999). The stimulated sites show increased RANK-RANKL binding and macrophage colony stimulating factor levels and decreased OPG concentration which indicates increased osteoclasts activation, production and activity (H. Huang et al., 2014). There is also increased expression of prostaglandins and cytokines such as PGE2, IL-1 and IL-6 which promotes osteoclastic activity and hastens the resorption process, reduces the duration of clearance of necrotic material (rate limiting step) and results in faster orthodontic tooth movement (H. Huang et al., 2014). The growth factors, cytokines and hormones that regulate osteoblast proliferation, differentiation and function are also increased which causes the increased bone formation rates (H. Huang et al., 2014).

2.2.1 How Does MOP Induce RAP?

Orthodontic tooth movement by itself is a noxious stimuli capable of inducing RAP for faster tooth movement (Melsen, 2001). By applying another noxious stimuli like selective alveolar decortication or MOP, the effect of RAP is increased and there is more rapid orthodontic tooth movement (Baloul et al., 2011; Tsai et al., 2016). Orthodontic force loading together with decortication resulted in an initial reduction of bone volumetric fraction or bone volume over total volume (BV/TV) which later recovered back to baseline values. However alveolar decortication alone did not cause any significant change to BV/TV values (Baloul et al., 2011). This indicates that alveolar decortication or MOP only enhances the rate of tooth movement by increasing bone turnover (Baloul et al., 2011), and a constant force is needed for initiating the coupled bone remodelling response (Baloul, 2016; Krishnan & Davidovitch, 2006, 2009).

The rate of orthodontic tooth movement is characterised into initial phase, lag phase and then continuous tooth movement phase. With RAP stimulation, the peak rate of

orthodontic tooth movement is reached much earlier and is maintained for a longer duration (Baloul et al., 2011). The RAP effect of alveolar decortication is also reported to be able to bypass the lag phase by increasing more rapid bone remodelling subsequently faster elimination of the hyalinization associated with the lag phase (Baloul et al., 2011). Besides increasing bone turnover, RAP also induces a transient localised reduction in bone volumetric fraction, similar to an osteoporosis state, which is thought to accelerate orthodontic tooth movement through bone (Goldie & King, 1984; Schilling, Muller, Minne, & Ziegler, 1998). In another study (Tsai et al., 2016), it was shown that MOP can induce statistically similar results for rate of tooth movement, BV/TV and bone mineral density when compared to alveolar decortication. This means that the increased bone turnover rate can be induced by different methods and MOP is one of them. MOP as a means of accelerating tooth movement was also investigated in a human study where it was shown that MOP significantly increased the rate of orthodontic tooth movements (M. Alikhani et al., 2013).

2.3 What Is Bone Volumetric Fraction (BV/TV)?

Alveolar bone trabeculae is the functional unit of cancellous bone and is also the main factor influencing the quality of trabeculae bone (Friedman, 2006; Fyhrie, 2005). This is due to the high bone turnover rate of trabeculae bone which influences bone healing (Minkin & Marinho, 1999; Sakka & Coulthard, 2009).

Bone volume fraction or Bone Volume over Total Volume (BV/TV) is the ratio of volume of mineralised bone to volume of the bone being investigated (Baloul et al., 2011). It refers to the trabeculae bone microstructure parameter and does not include cortical bone volume (Kazama et al., 2010). BV/TV is the most commonly used output of micro-

CT analysis performed on bone. It can be used to evaluate relative changes in bone volume density following a given treatment (Legrand et al., 2000).

BV/TV or trabecular bone microstructures properties also differ throughout the skeleton and vary from individual to individual. However, the BV/TV values are quite similar for similar sites throughout the skeleton (Eckstein et al., 2007). Low BV/TV values indicate reduction in trabecular bone microstructure or reduced bone density (Teixeira et al., 2010; Tsai et al., 2016). RAP will increase bone remodelling with accompanying increased osteoclast and osteoblast activity which presents as reduction in trabecular bone microstructure due to formation of less dense, less mineralised osteoid. This will show up in the computed tomography analysis as a reduction in BV/TV (Baloul et al., 2011).

2.4 Why Measure BV/TV?

Previous CBCT human studies on orthodontic tooth movement had mainly assessed bone mineral density changes (Chang et al., 2012; da Silva Campos et al., 2012; Hsu et al., 2011; Yu et al., 2016). No human studies on bone volume fraction changes associated with accelerating orthodontic tooth movement have been reported. Whereas almost all animal studies on acceleration of orthodontic tooth movement with corticotomy or MOP had reported both bone mineral density and bone volume fraction changes (Baloul et al., 2011; Librizzi et al., 2017; C. A. Murphy et al., 2014; Sugimori et al., 2018; Tsai et al., 2016). This is because both changes in overall bone structure (represented by BV/TV) and mineral content (bone mineral density) reflect the coupled bone remodelling cycles induced by alveolar decortication. Hence, it is prudent to measure both parameters for better representation of the bony change (Baloul et al., 2011).

CBCT has been reported to be weak in measuring bone density level (Campos, de Souza, Mota Junior, Fraga, & Vitral, 2014; Cassetta, Stefanelli, Pacifici, Pacifici, & Barbato, 2014) but on par with micro-CT (Ibrahim et al., 2014; Parsa, Ibrahim, Hassan, van der Stelt, & Wismeijer, 2015), which is the gold standard for BV/TV measurements. Besides that, high bone density does not always correspond to high trabeculae parameters such as trabeculae number and trabeculae thickness as bone quality is dependent on four factors, namely microstructure, density, bulk and spacing (Gomes de Oliveira, Leles, Lindh, & Ribeiro-Rotta, 2012). Based on these facts, it would be a better choice to use CBCT measured BV/TV as the parameter to represent bone changes associated with MOP accelerated orthodontic tooth movement.

The investigation of bone volumetric fractions through a non-invasive, non-destructive means like CBCT allows for representative observation of the bone homeostasis effects of MOP (Baloul et al., 2011). Histological studies have shown that there is bone resorption on the pressure side and bone deposition on the tension side in relation to orthodontic force application (Krishnan & Davidovitch, 2006). This can be observed as BV/TV reduction for bone resorption and BV/TV recovery or increase for bone deposition (Baloul et al., 2011). The mechanism of action of RAP following a mechanical injury (such as MOP) is the coupling of osteoclasts and osteoblast in the same time frame following noxious stimuli (Sebaoun et al., 2008). The overlap of resorptive and formative changes in the bone creates a dynamic environment (King, Keeling, & Wronski, 1991) that accelerates the turnover rate of alveolar bone which increases orthodontic tooth movement (Verna et al., 2000). Animal studies of micro-CT investigations of bone volume fractions show earlier, more and longer lasting reduction of BV/TV occurring in orthodontic tooth movement with selected alveolar decortication groups compared to just orthodontic tooth movement alone (Baloul et al., 2011; Sugimori et al., 2018; Tsai et al.,

2016) thus proving the mechanism of action of RAP. These studies prove that BV/TV is a viable parameter for analysing trabeculae bone pattern changes associated with MOP.

2.5 Methods to Measure BV/TV

Trabeculae bone microstructures are three-dimensional structures which must be evaluated with three-dimensional imaging techniques. According to a series of articles by Genant et al (Genant et al., 1996; Genant, Engelke, & Prevrhal, 2008; Genant et al., 2000), non-invasive, non-destructive imaging techniques such as computed tomography (CT) are reliable methods for analysing trabecular micro-structure density. The CT detects minerals which are the densest elements in alveolar bone and is measured in Hounsfield units (HU). CT can be divided into in-vivo use (high-resolution CT, volumetric quantitative CT, high-resolution magnetic resonance) or in-vitro use (micro-CT and micro magnetic resonance) based on their radiation exposure.

2.5.1 Micro-CT

High resolution micro-CT is used for assessing various bone parameters such as bone mass, bone density and bone trabeculae microstructure. It provides detailed and accurate bone trabeculae patterns due to its ability to attain isotropic voxel size of almost 10 µm (Faot, Chatterjee, de Camargos, Duyck, & Vandamme, 2015). The width of rodent trabeculae bone ranges from 50-100 µm, thus voxel size of 10-20 µm is required for accurate microstructure assessment which is achievable with micro-CT (Muller et al., 1996). Micro-CT has been recommended as a gold standard imaging for ex-vivo bone studies due to its high resolution (Yip, Schneider, & Roberts, 2004). This is evident in the literature as many animal studies used micro-CT to analyse BV/TV changes (M. Alikhani

et al., 2012; An, Li, Liu, Wang, & Zhang, 2017; Baloul et al., 2011; Cheung et al., 2016; Sugimori et al., 2018; Teixeira et al., 2010; Tsai et al., 2016; Xu, Zhao, Xu, & Ding, 2013; Zhuang, Bai, & Meng, 2011). The radiation dose of a micro-CT scan is very high and can even influence the experimental outcome (Willekens et al., 2010) and is detrimental for human evaluation purpose. Despite all its effectiveness in providing high resolution CT images, micro-CT is limited to small animal studies due to its high radiation dose and limited scanning range.

2.5.2 Cone Beam Computed Tomography (CBCT)

CBCT is a three-dimensional imaging modality developed in the 1990s to replace single and multi-slice CT for diagnostic imaging in oral implants (Hatcher, 2010). Due to its many advantages such as rapid scan and processing time, high resolution images, relatively low radiation doses and cost, CBCT has superseded other CT imaging modalities in dentistry (Scarfe, Farman, & Sukovic, 2006). Despite all its advantages in clinical application of CBCT, it was reported that CBCT was lacking in measurement of bone density due to differing grayscale density values (Cassetta et al., 2014) and artefacts (Molteni, 2013; Schulze et al., 2011). Campos et al also discouraged the use of CBCT for measurement of bone mineral density due to inconsistencies in gray values related to object density, scattering effect of radiation beam and x-ray beam hardening effect (Campos et al., 2014).

Recent studies have reported good reliability of CBCT as a tool to measure bone volumetric fraction or BV/TV. Two studies (Gonzalez-Garcia & Monje, 2013; Van Dessel et al., 2013) in comparing CBCT against micro-CT in assessing BV/TV of bone biopsies, reported good reliability in favour of CBCT. Another study reported strong correlation between bone morphometric parameters analysed by CBCT and two-

dimensional histology (Y. Huang et al., 2014). A more recent study concluded that the bone quality related morphometric indices (including BV/TV) measured by CBCT were at a level of accuracy and reliability near approaching to that of micro-CT (Van Dessel et al., 2017).

CBCT imaging modalities have been used in orthodontics mainly for analysing bone density (Chang et al., 2012; da Silva Campos et al., 2012; Hsu et al., 2011; Yu et al., 2016). All four studies report that bone density was reduced with orthodontic tooth movement and that CBCT is a useful modality in evaluating bone density changes. Yu et al evaluated the bone density changes throughout the course of orthodontic treatment and reported that the alveolar bone density around teeth was reduced during active orthodontic tooth movement and the bone density levels returned to pre-treatment values after the retention period (Yu et al., 2016).

2.5.3 How Does CBCT Work?

CBCT is a type of three-dimensional (3D) volumetric imaging technology. It consists of an x-ray source which produces a cone shaped ionizing radiation beam and an x-ray detector to capture the image. Both x-ray source and detector rotate around the center of the field of view (FOV). During the rotation, multiple (150-600) sequential image projections are acquired. These stack of images are stacked together by a computer to reconstruct a three-dimensional (3D) representation. As the CBCT exposure encompasses the entire FOV, only one rotation is needed to obtain the data for image construction (Scarfe & Farman, 2008).

The image reconstruction process can be divided into 3 stages (Whaites & Drage, 2013):

i. Data acquisition – the CBCT machine scans the patient
ii. Primary reconstruction – the scan is divided by the computer into voxels and calculates the x-ray absorption in each voxel and allocated a gray shade that ranges from black (maximum x-ray absorbed by detector) to white (zero x-rays absorbed)
iii. The computer software constructs a multi-planar image

The resolution of a CBCT image is determined by the voxels or individual volume elements. Voxel dimensions are dependant on pixel size on the x-ray detector. The resulting CBCT images consist of submillimeter isotropic voxel resolution ranging from 0.076 – 0.4 mm, thus providing accurate multi-planar reformation images (Scarfe & Farman, 2008).

CBCT radiation doses varies depending on the machine, field of view (FOV), and technique (Ludlow, Davies-Ludlow, Brooks, & Howerton, 2006). The effective dose of a CBCT is about 4 – 42 time greater than a panoramic radiograph (Ludlow et al., 2006). The mean adult effective dose based on size of FOV were 212 μSv for large field of view, 177 μSv for medium field of view and 84 μSv for small field of view (Ludlow et al., 2015).

2.6 MOP Effects on BV/TV

2.6.1 Range of BV/TV Reduction after MOP

According to Midgett, the greatest orthodontic tooth movement occurs in alveolar bone with loose trabeculae and less bone resistance (Midgett, Shaye, & Fruge, 1981). This

statement has been proven partly by the various human (M. Alikhani et al., 2013) and animal studies (Baloul et al., 2011; Cheung et al., 2016; Sugimori et al., 2018; Teixeira et al., 2010; Tsai et al., 2016) that report significant increase in orthodontic tooth movement with MOP or corticotomy intervention. The animal studies (Teixeira et al., 2010; Tsai et al., 2016; Verna et al., 1999) also describe that the RAP effect is not just limited to the area of intervention, but also extended to the surrounding adjacent teeth (but not to the contralateral side) as a reduction in bone density and BV/TV. However, to date no human studies have reported on the extent of BV/TV reduction following MOP intervention. This information is clinically relevant as it would provide a clinician with the effective range of a MOP intervention thus allowing proper planning of the location and number of MOP needed to induce the most effective orthodontic tooth movement.

2.6.2 Duration of BV/TV Reduction after MOP

Following MOP in rodents, Tsai et al reported an insignificant reduction of BV/TV. However the changes are significant for reduction of bone mineral density, faster tooth movement after 3 weeks; BV/TV and bone mineral density reduction and faster tooth movement after 6 weeks (Tsai et al., 2016). Based on the results of their study, they concluded that RAP was observed within 2 weeks of MOP intervention. Buschang et al applied the RAP duration for dogs (Cohen, Campbell, Rossouw, & Buschang, 2010) to humans and hypothesized that the RAP duration in humans lasts about 2-3 months (Buschang, Campbell, & Ruso, 2012). RAP is initiated within a few days following noxious stimuli (MOP), then increases and peaks in 1-2 months before subsiding back to normal levels about 4 months later (Amit, Jps, Pankaj, Suchinder, & Parul, 2012; Schilling et al., 1998; Wilcko et al., 2001). Baloul et al reported that following selective alveolar decortication in rodents, BV/TV was significantly reduced at 7 days then slowly

recovered back to normal levels at 28 days (Baloul et al., 2011). However, there are no human studies that report the BV/TV pattern changes in relation to MOP intervention. In addition, no studies have reported the trabeculae pattern changes following multiple subsequent RAP inducing surgical interventions. Localized increased in turnover rate of alveolar trabeculae is induced by MOP intervention (Sebaoun et al., 2008; Tsai et al., 2016). As the RAP effect has a peak duration, would multiple MOP interventions be able to stimulate the BV/TV to be maintained close to peak levels thus extending the duration of RAP?

2.7 Clinical Application of MOP

In a clinical guide for MOP application (Chinapa Sangsuwon, Alansari, Lee, Nervina, & Alikhani, 2017), it was recommended that MOP should be placed based on the location, number and depth of the MOP. For location, the recommended protocol was to apply MOPs around the target tooth, focusing the MOP in the desired direction of tooth movement. MOP was also advocated to be placed more apically to accelerate orthodontic tooth movements that require more root movements such as intrusion and torque. Furthermore, the placements of MOPs were advised to be limited to the attached gingiva and proximal to tooth roots. The basis of these recommendation were based on the research that MOPs stimulates catabolic and anabolic (remodeling) effects in bone (Mani Alikhani et al., 2015; M. Alikhani et al., 2013; C. Sangsuwon, Alansari, Nervina, Teixeira, & Alikhani, 2017; Teixeira et al., 2010). The catabolic effects are useful in the following clinical scenarios such as acceleration of orthodontic tooth movement, uprighting teeth, tracking unerupted teeth and torqueing movements (Mani Alikhani et al., 2015). However, there were no references as to the extent of these catabolic effects. The first study on MOP effect in humans concluded that MOPs can significantly

accelerate orthodontic tooth movement, and that future studies on number and frequency of MOPs be conducted to facilitate clinical application (M. Alikhani et al., 2013). A recent study had describe that the overall canine retraction was not affected by different MOP intervals (Sivarajan et al., 2018). To date no study has investigated the spread of mandibular trabeculae pattern in relation to different MOP intervals for accelerating orthodontic tooth movement. This evidence could serve as further useful guide to the actual application of MOP clinically.

CHAPTER 3: METHODOLOGY

3.1 Trial Design

This was a prospective randomized single centre clinical trial conducted at the Faculty of Dentistry, University of Malaya. Comprising 2 parts, the first stage was a clinical split-mouth study where comparison of tooth movement (canine retraction) was done between the intervention (MOP) side of the lower arch and conventional canine retraction on the contralateral side. The later stage of the trial was aimed to analyse the change in the trabecular bone volumetric fraction (BV/TV) using cone beam computed tomography (CBCT). Both stages were conducted by different orthodontic postgraduate students, SS and TNHK respectively.

3.2 Sample Size Calculation

Our study was to evaluate trabecular bone volume fraction changes in the mandible between the MOP intervention and the conventional canine retraction sides, and to correlate this to the canine retraction rate. A power analysis was calculated with G*Power: Statistical Power Analyses software (Faul, Erdfelder, Lang, & Buchner, 2007). We used the BV/TV means of 46.02 ± 5.60% (MOP) and 59.48 ± 7.53% (Control) from an animal study (Tsai et al 2016) to estimate the effect size because there is as yet, no human studies reported to estimate BV/TV values. To be able to observe an effect size of 0.7 at the $\alpha = 0.05$ significance level and to have an 80% power to detect significant differences within 3 groups, a sample size of 24 patients divided into 8 subjects per group in a 1:1:1 ratio would be needed. To account for dropouts, a total sample size of 30 participants were recruited.

As this is a split mouth study, the sample size of 24 subjects with 2 observation sites per patient would be equivalent to 48 sites, with each patient contributing 2 sites (Pandis, 2012).

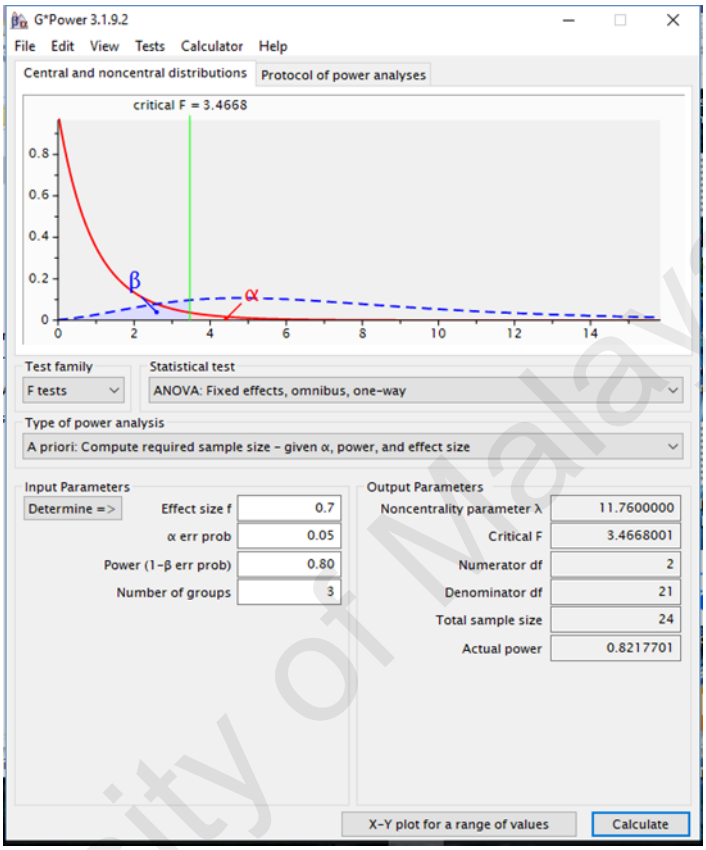

Figure 3.1 Sample size calculation using G*Power software

3.3 Participants, Eligibility Criteria and Settings

Participants for the trial was recruited from the Dental Clinic at the Faculty of Dentistry, University of Malaya and treated at the Postgraduate Orthodontic Clinic, Faculty of Dentistry, University of Malaya by one orthodontic postgraduate student (SS) under the supervision of one orthodontic Consultant (WMC), who was the main supervisor of this study. The total sample size collected for the purpose of this trial was 30 participants. Eligibility criteria is as displayed below:

Table 3.1 Eligibility Criteria for the study

ELIGIBILITY CRITERIA FOR THE STUDY	
INCLUSION CRITERIA	EXCLUSION CRITERIA
Age range : 18-years-old and above	
Either molar Class I malocclusion or molar Class II or III < ½ unit Requiring extraction of all four permanent first premolar teeth Maximum anchorage control with temporary anchorage device (TAD)	
All permanent teeth erupted from upper right first molar to upper left first molar, lower right first molar to lower left first molar.	Impacted teeth Unerupted teeth Missing teeth
Average vertical facial proportions	Vertical skeletal discrepancies, eg. high angle and low angle
No temporal mandibular joint related problems	History or present temporal mandibular joint related problems
No systemic disease	Systemic disease especially on long term use of antibiotics, phenytoin, cyclosporine, anti-inflammatory drugs, systemic corticosteroid and calcium channel blockers
Good oral hygiene	Poor oral hygiene for more than 3 visits
No history of periodontal therapy	Past periodontal disease
No current active periodontal disease	Current periodontal disease

3.4 Ethical Approval

Ethical approval for the conduct of this study was obtained from the Ethics Committee of the Faculty of Dentistry, University of Malaya (DF CD1412/0089(P)). All patients who match the inclusion criteria were informed of the trial and offered to participate in the trial. Informed consent was obtained from voluntary participants, and patients who declined participation were treated as normal patients at the Postgraduate Orthodontic Clinic in Faculty of Dentistry, University of Malaya.

3.5 Randomisation

Of the total of 30 patients recruited for the trial, each patient was assigned a specific research code.

The following characteristics had to be randomly assigned to each trial participant.

i. Intervention and control side for the mandible
ii. Intervention interval for the mandible

There were 3 groups of different intervals:

i. Group 1 – 4 weekly intervention interval in the mandible.
ii. Group 2 – 8 weekly intervention interval in the mandible.
iii. Group 3 – 12 weekly intervention interval in the mandible.

First, the 30 participants were randomly assigned to one of the 3 intervention interval groups at a 1:1:1 distribution, by matching their research code to the simple randomized block sampling sequence generated online at RANDOM.ORG. Following that, simple randomised block sampling for the intervention and control side for the mandible was done separately via drawing of lots by the patients themselves. The following steps described the randomisation process.

i. Patients randomly assigned to interval groupings, groups 1, 2 and 3
ii. Patient drew lots to randomise intervention and control sides of mandible

3.6 Blinding

Randomisation, treatment, intervention and data collection were carried out by the orthodontic postgraduate students (SS and TNHK) and the orthodontic consultant

(WMC), thus it was not possible to blind the clinicians and patients to the intervention and control side, intervention interval and data collection in the clinical part of the study. However the CBCT data of the patients were allocated codes which served to blind the orthodontic postgraduate student (TNHK) who analyzed the trabecular bone volumetric fraction data. This blinding ensured some partiality and reduced bias during data analysis.

3.7 Interventions

The first clinical stage of this trial was done by one postgraduate student (SS) assisted by an orthodontic consultant (WMC). At the first appointment, preadjusted edgewise brackets (3M 0.022" x 0.028" slot in MBT prescription) were bonded from the second premolar to the contralateral second premolar of all quadrants, except the first premolars which were scheduled to be extracted. Elastomeric separators were placed at all the first molars.

At the second appointment, the first molars were banded (3M 0.022" x 0.028" slot in MBT prescription) and separators then placed at the second molars. Following that, appointments were arranged for banding of second molars, then insertion of mini-implants with accompanying indirect anchorage in each quadrant, and then extraction of all first premolars in each quadrant.

A mini-implant (Orlus Extra thread Mini-Implant, 1.6mm width, 6mm length) was placed in the keratinised gingiva between the second premolars and first molars of each quadrant, standardised at 3mm apical to the cervical margin of the second premolar. Dislodged mini-implant was reinserted 5mm apical to the cervical margin of the second premolar. The gingiva was anaesthetised (Scandonest 2%, 2.2ml containing 44mg Mepivacaine Hydrochloride; 22 microgram Adrenaline) prior to mini-implant insertion,

and guided via periapical radiographs and an insertion jig made with 0.020" Australian stainless steel. Subsequently, a coiled loop bent from 0.020" Australian stainless steel extending from mesial of the first molar bands to the distal surface of the mini-implant was placed to serve as indirect anchorage to ensure absolute anchorage of the first molars.

With the anchorage of the first molars secured, the first premolars were then arranged to be removed, the sequence scheduled according to the quadrant where anchorage preservation was deemed more demanding (eg, crowding and centreline shift). Both extractions and placement of mini-implants were completed within 1 month after bonding.

Following first premolar extractions, a standardised arch wire sequence was used, comprising of

i. 0.014" Nickel Titanium (3M Nitinol SuperElastic),
ii. 0.018" Nickel Titanium (3M Nitinol SuperElastic),
iii. 0.017" x 0.025" Nickel Titanium (3M Nitinol SuperElastic) and
iv. 0.018" x 0.025" Stainless Steel (GAC PAK Stainless Steel ACCUFORM).

The interval between each archwire change was 6 weeks. Elastomeric bumper sleeves were used to maintain extraction spaces, prior to canine retraction.

Canine retraction was done 1 month after the 0.018" x 0.025" stainless steel working archwires were placed. All 4 canine teeth were retracted using power chain (3M Unitek Alastik Chain) loaded with 140-200g force, and stretched from canine brackets to the mini-implants of the respective quadrants. Micro-osteoperforations (MOP) were carried out at intervention sites and none at control sites.

The sites chosen for MOP was at the buccal cortical bone, equidistance from the canine and second premolar tooth. Prior to MOP at the intervention sites, the gingiva was anaesthetised (Scandonest 2%, 2.2ml containing 44mg Mepivacaine Hydrochloride; 22 microgram Adrenaline) and the thickness of the attached gingiva measured with a Williams periodontal probe. Three MOPs of 3mm depth into bone and 2mm apart from each other were placed in a vertical line at the buccal cortical bone through the gingiva, approximately at the horizontal level of the cervical margin of the canine tooth and extending apically. A rubber stopper was used to mark the sum of 3 mm to the gingiva thickness on the mini-implant (Orlus Extra thread Mini-Implant, 1.6mm width, 6mm length). This way, the depth of MOP was standardized to 3mm in buccal cortical bone. Subsequently, pressure with cotton pellet was used to achieve haemostasis. Paracetamol 1000mg was prescribed for pain management on an "only when needed" basis.

All 3 groups had MOP at the intervention sites at the start of the 16 weeks, which coincide with the start of canine retraction. Patients were reviewed every 4 weekly over a period of 16 weeks. At these appointments, the distance of canine movement was recorded clinically with digital callipers with an accuracy of 0.01mm, the power chains for canine retraction were replaced, and MOP performed according to the scheduled intervals. This meant that Group 1 (4 weekly intervention interval in the mandible) had a total of five MOPs within the 16 weeks' period; Group 2 (8 weekly intervention interval in the mandible) only had three MOP within the 16 weeks' period and Group 3 (12 weekly intervention interval in the mandible) had two MOP within the 16 weeks' period (Table 3.2).

3.7.1 CBCT Data Collection

At 12 weeks after start of canine retraction, a CBCT image of the left and right mandibular quadrant (both control and intervention sides) extending from the lateral incisor to the first molar was done before receiving clinical treatment for that appointment. The patients were then followed up until 16 weeks and the rate of canine movement measured accordingly. After that, all patients were treated to completion as determined in the treatment plan and eventually debonded.

As the CBCT image was taken at the 12th week review, the MOP done on the same day would not have expressed their effect. Hence the CBCT would only record the effects of three MOP interventions for Group 1 (4 weekly intervention interval in the mandible), two MOP interventions for Group 2 (8 weekly intervention interval in the mandible) and one MOP intervention for Group 3 (12 weekly intervention interval in the mandible). This was the reason for taking the CBCT image at the 12th week review instead of the 16th week review.

Table 3.2 MOP Intervention and CBCT schedule

CBCT scan ↓

	Start of canine retraction	4th week review	8th week review	12th week review	16th week review
Group 1 (MOP every 4 weeks)	MOP	MOP	MOP	MOP	MOP
Group 2 (MOP every 8 weeks)	MOP		MOP		MOP
Group 3 (MOP every 12 weeks)	MOP			MOP	

3.7.2 CBCT analysis

During the second stage of the trial, CBCT dataset was processed to analyse the trabecular bone volumetric fraction (BV/TV) of control and intervention sites was done by researcher TNHK. The CBCT images of the left and right side of the mandible was taken using Kodak 9000C 3D model CBCT machine with the scanning exposure settings of 70kV, 10mA, 10.80 seconds, field of view (FOV) of 5cm x 5cm and voxel size of 76µm. As the CBCT imaging was done digitally, the accompanying software from Kodak, the Trophy DICOM version 6.4.0.4 was used to reconstruct the 3D radiographic image and subsequently saved as a set of DICOM® (Digital Imaging and Communications in Medicine) files. ImageJ (version 1.50i, a public domain image processing software) (Figure 3.2) was then used to convert the DICOM® files into a series of BMP (bitmap image) files (Figure 3.3), which were then analysed using the CT Analyser program (version 1.11.0.0 copyright SkyScan) (Figure 3.4). The analysis of BV/TV parameter were performed using the CT Analyser program. This allowed for the three-dimensional mapping of the trabecular bone pattern and the calculation of the trabecular bone volumetric fraction.

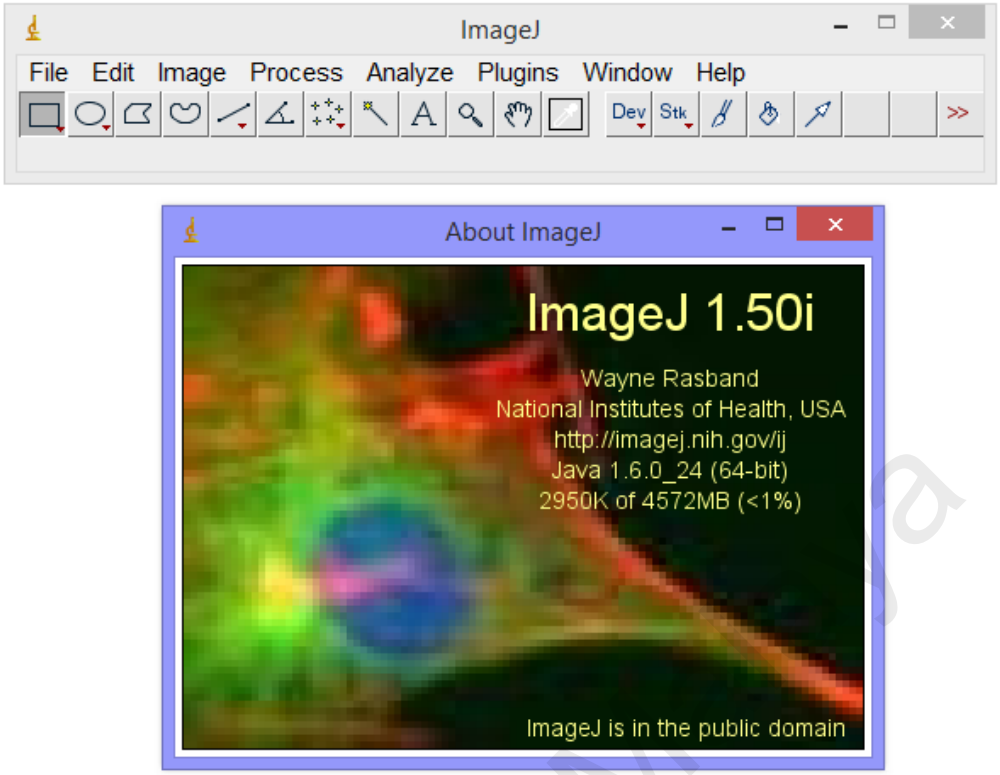

Figure 3.2 Image J version 1.50i image processing software

Figure 3.3 Series of BMP (bitmap image) files

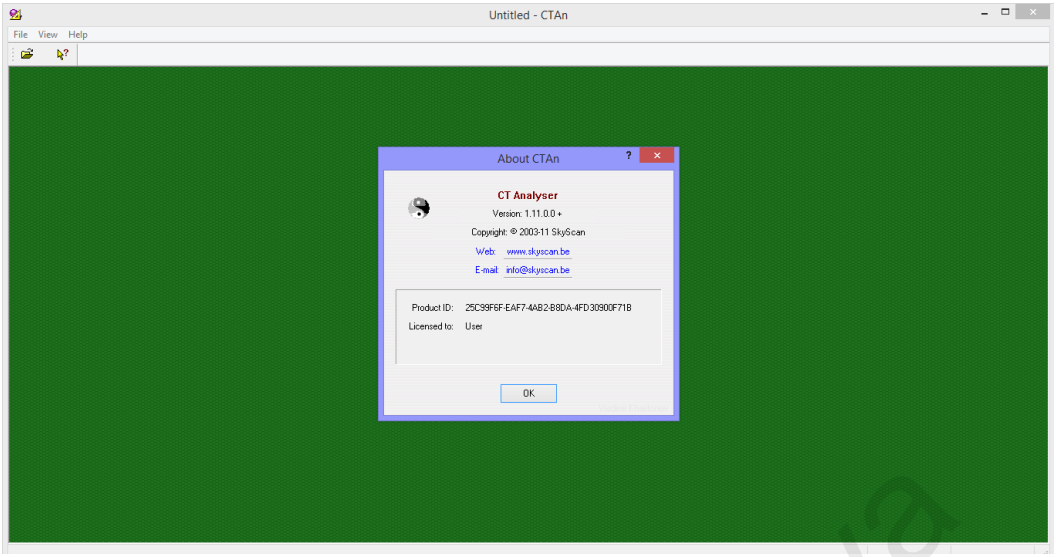

Figure 3.4 CT Analyser program version 1.11.0.0 from SkyScan

Each mandibular quadrant was divided into vertical and horizontal region of interest (ROI) for analysis. The vertical ROI consisted of the trabeculae bone between the distal surface of the mandibular canine tooth root to the mesial surface of the mandibular second premolar root (Figure 3.5). The top slice of the ROI started from a slice apical to the cemento-enamel junction (CEJ) of the canine tooth, while the bottom slice of the ROI was determined to be the slice 44.4mm apical to the upper slice on the axial view of the CBCT image. The lateral borders of the vertical ROI were the distal cortical plates of the canine tooth socket and the mesial cortical plates of the first molar tooth. The vertical ROI was then divided into three equal vertical sections of 14.8mm in height where the top part was labelled as V1, the middle as V2 and the apical third as V3. The height of each vertical section was determined at 14.8mm to account for the sum of the diameter of 3 MOPs (1.6mm) and the distance of the spaces in between MOPs (the MOP sites are 2mm apart from each other and 3mm from the upper and lower border to account for any margin of error).

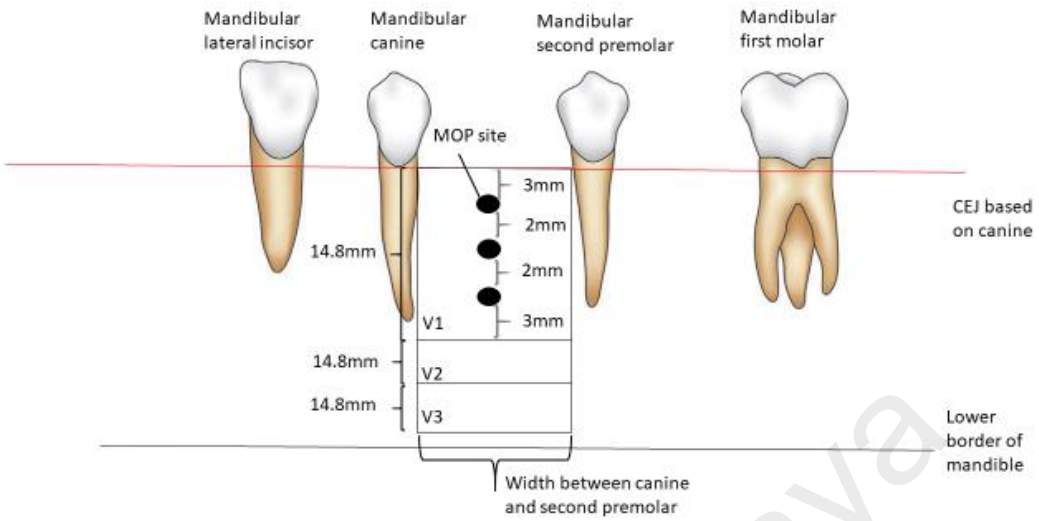

Figure 3.5 Schematic diagram for vertical sections

The horizontal ROI consisted of the trabeculae bone extending from the distal surface of the mandibular lateral incisor to the mesial surface of the mandibular first molar (Figure 3.6). The top border of the horizontal ROI was set at 7.4mm apical from the CEJ of the mandibular canine tooth. This upper limit enabled better analysis of available interdental trabeculae bone in between the apically tapering roots of the mandibular lateral incisor and canine, while staying at a vertical level that involved the MOP sites. The bottom limit of the horizontal ROI was set at 14.8mm from the CEJ of the mandibular canine. The horizontal ROI was divided into 3 unequal sections (H1-H3). H1 consisted of the trabeculae bone from the distal cortical plate of the mandibular lateral incisor tooth socket to the mesial cortical plate of the mandibular canine tooth socket. H2 extended from the distal cortical plate of the mandibular canine tooth socket to the mesial plate of the mandibular second premolar tooth socket. H3 consisted of trabeculae bone from the distal cortical plate of the mandibular second premolar tooth socket to the mesial surface of the mandibular first molar cortical plate.

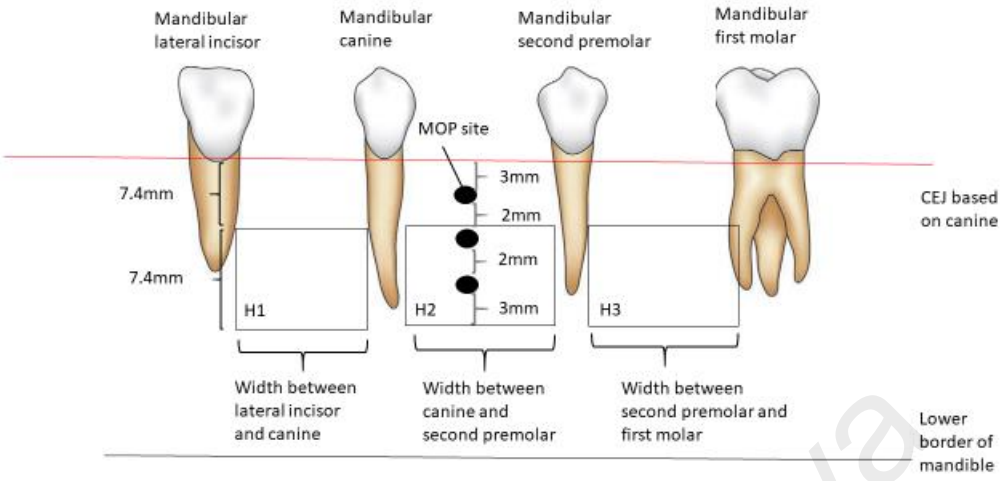

Figure 3.6 Schematic diagram for horizontal sections

Firstly, the BMP files of a single mandibular quadrant were exported to the CT Analyser program which then appeared in the "Raw images" tab as an image series of layers of mandibular structure with their associated thickness measurements (Figure 3.7).

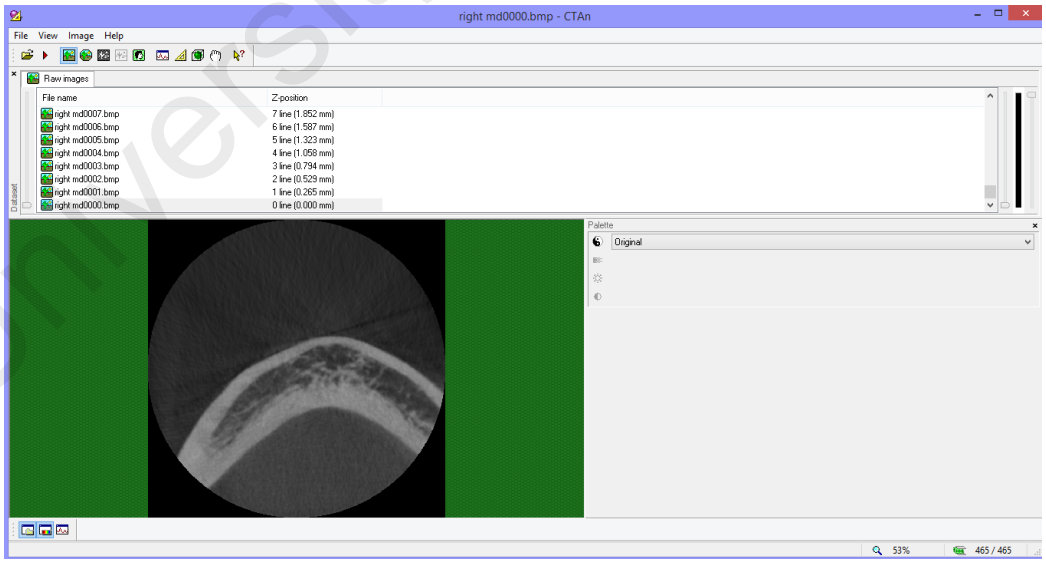

Figure 3.7 Raw tab of CT Analyser showing the BMP files loaded

Next, the layer containing the CEJ of the canine tooth was located (Figure 3.8). This layer is very important as it serves as the starting reference point for the analysis. In order to locate the CEJ of the canine, the crown of the canine was identified then followed apically until the layer where the radio-opaque mass of the crown immediately terminated, this layer was then noted as the CEJ layer which was then set as the top selection in the "Raw images tab" (Figure 3.8 - 3.10).

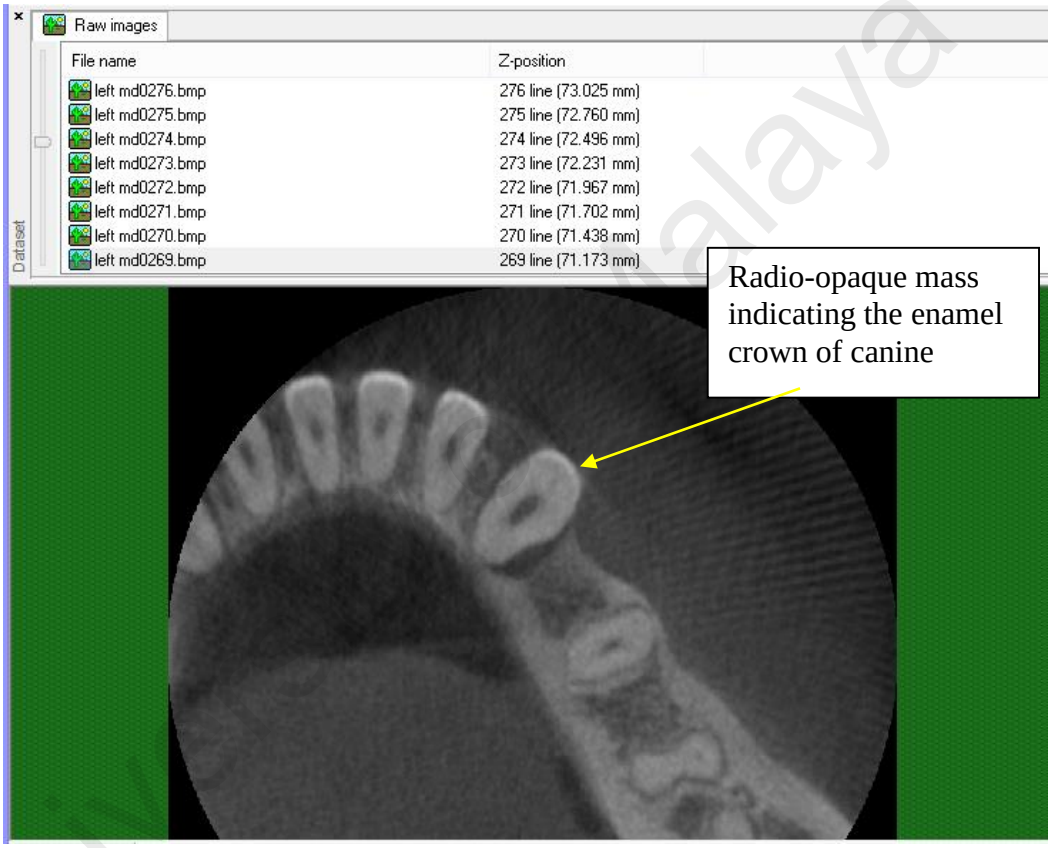

Figure 3.8 Layer showing crown of canine

Figure 3.9 Axial slice image showing cemento-enamel junction

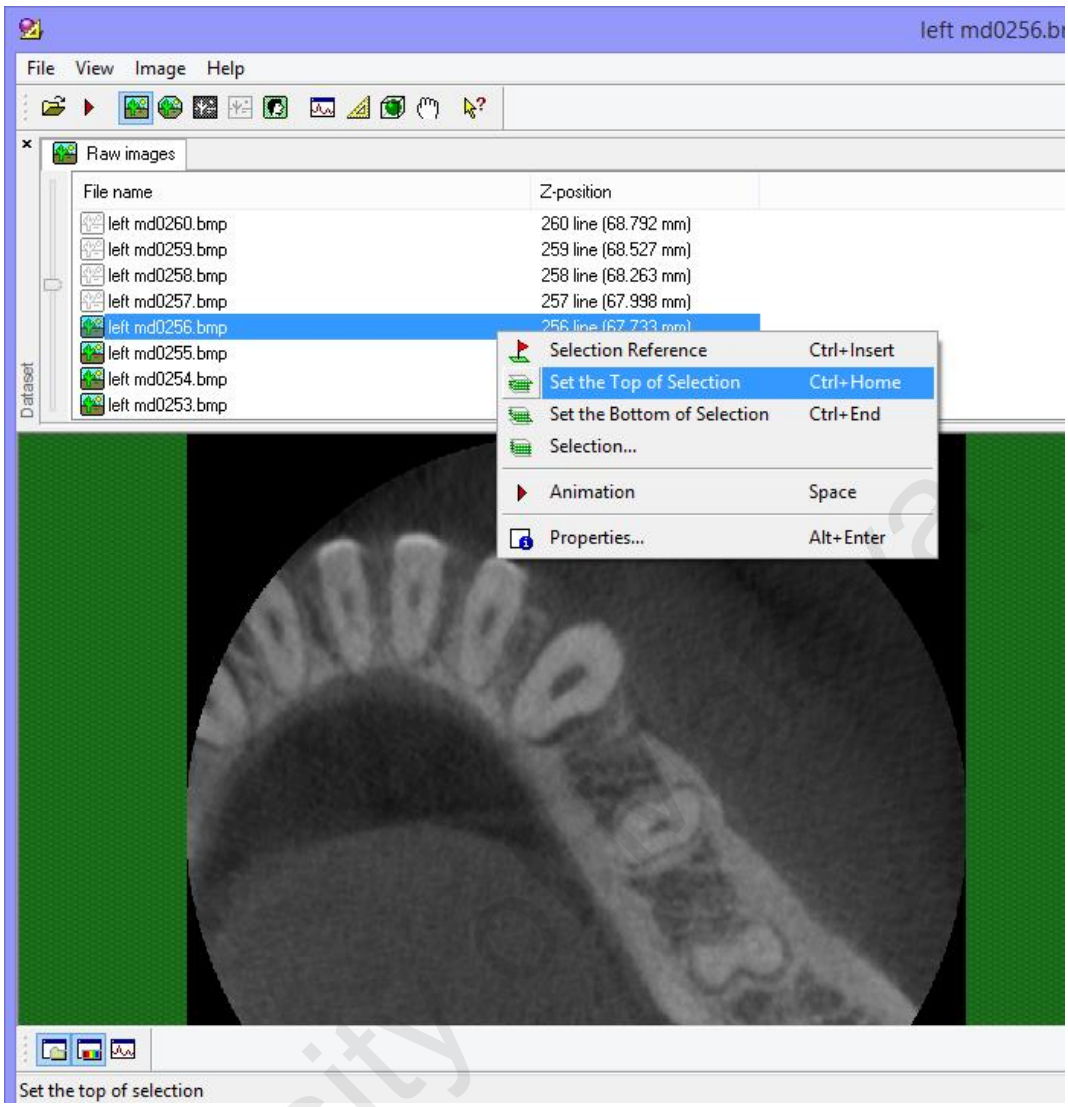

Figure 3.10 Setting the CEJ layer as the top layer of selection

The CEJ layer served as the top border of V1. The bottom border of V1 was determined by subtracting 14.8mm from the CEJ layer (67.733 mm) then set as the bottom selection (Figure 3.11).

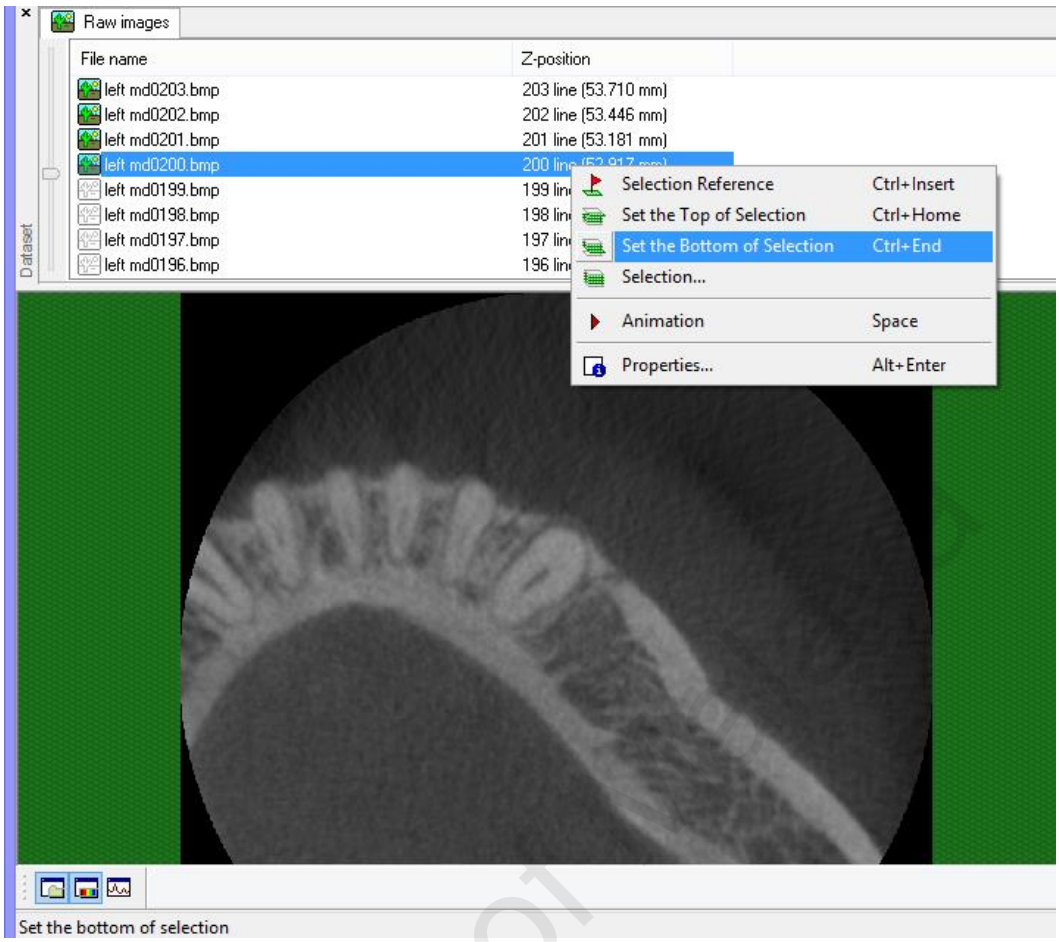

Figure 3.11 Setting the bottom layer of the region of interest (ROI)

The selected layers were then transferred to the "Region of interest" tab (Figure 3.12). A polygonal region of interest with editable points was used to mark the region of V1 at the top and bottom layers of selection (Figure 3.13 – 3.15). Following that, an adaptive interpolation was selected to have a better, more adapted selection of the region of interest (ROI) (Figure 3.16). The ROI was then checked manually at each individual layer to ensure correct selection of the region of interest. If the program erroneously selected the wrong region of interest on a particular layer, then a manual selection of the ROI was done instead.

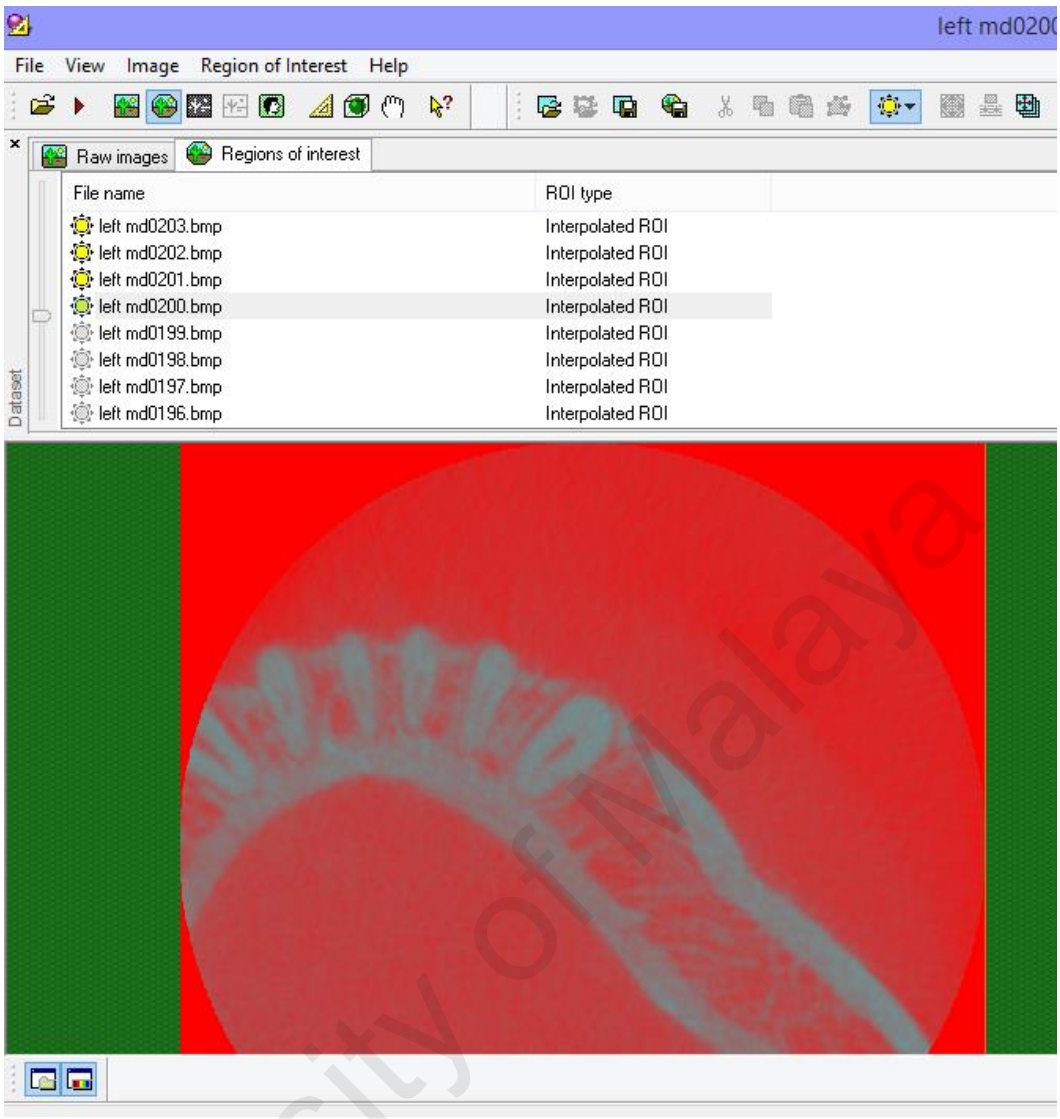

Figure 3.12 Region of Interest Tab

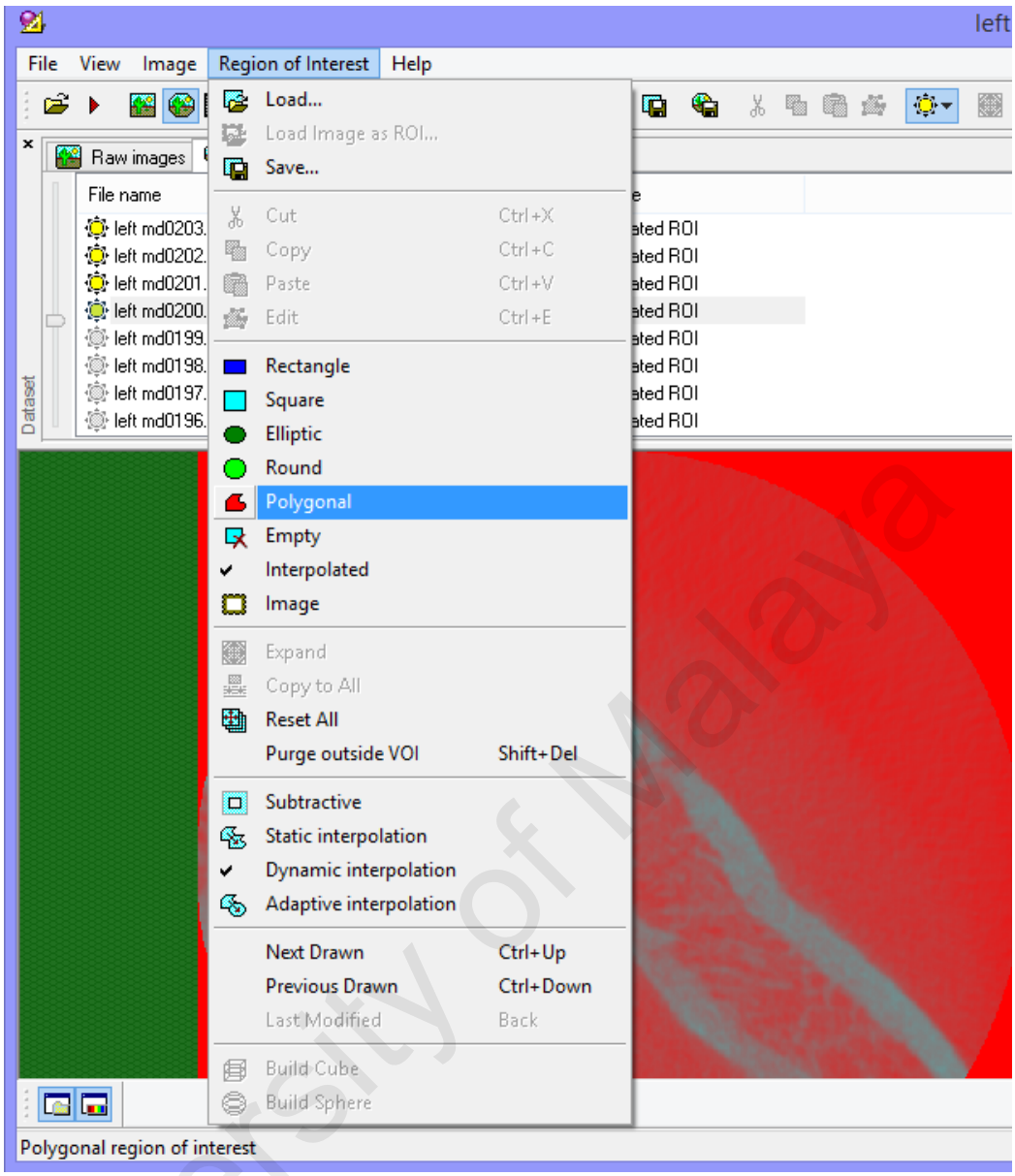

Figure 3.13 Polygonal shape for region of interest selection

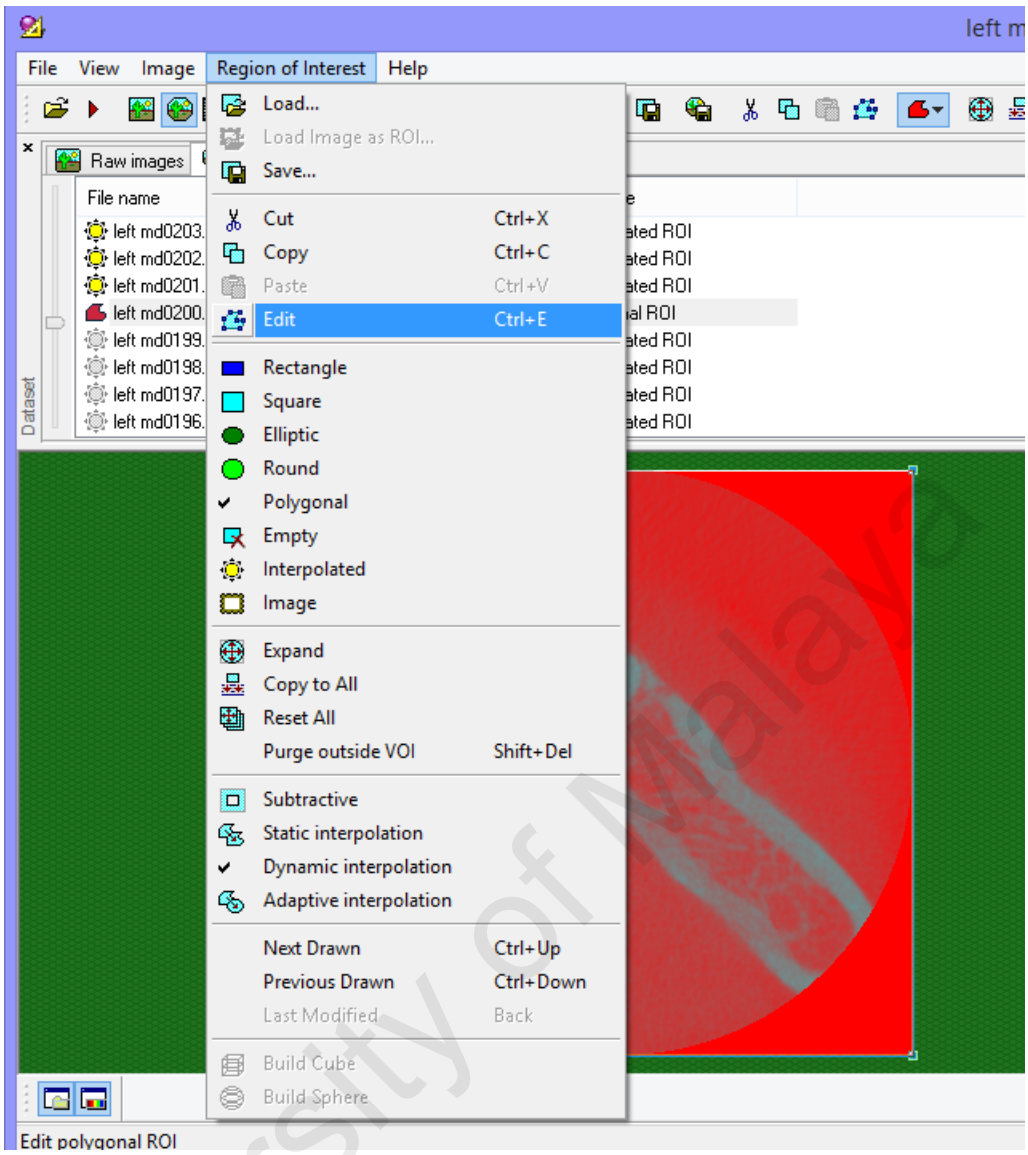

Figure 3.14 Editable polygonal shape for region of interest selection

Figure 3.15 The top layer selection for the region of interest of V1

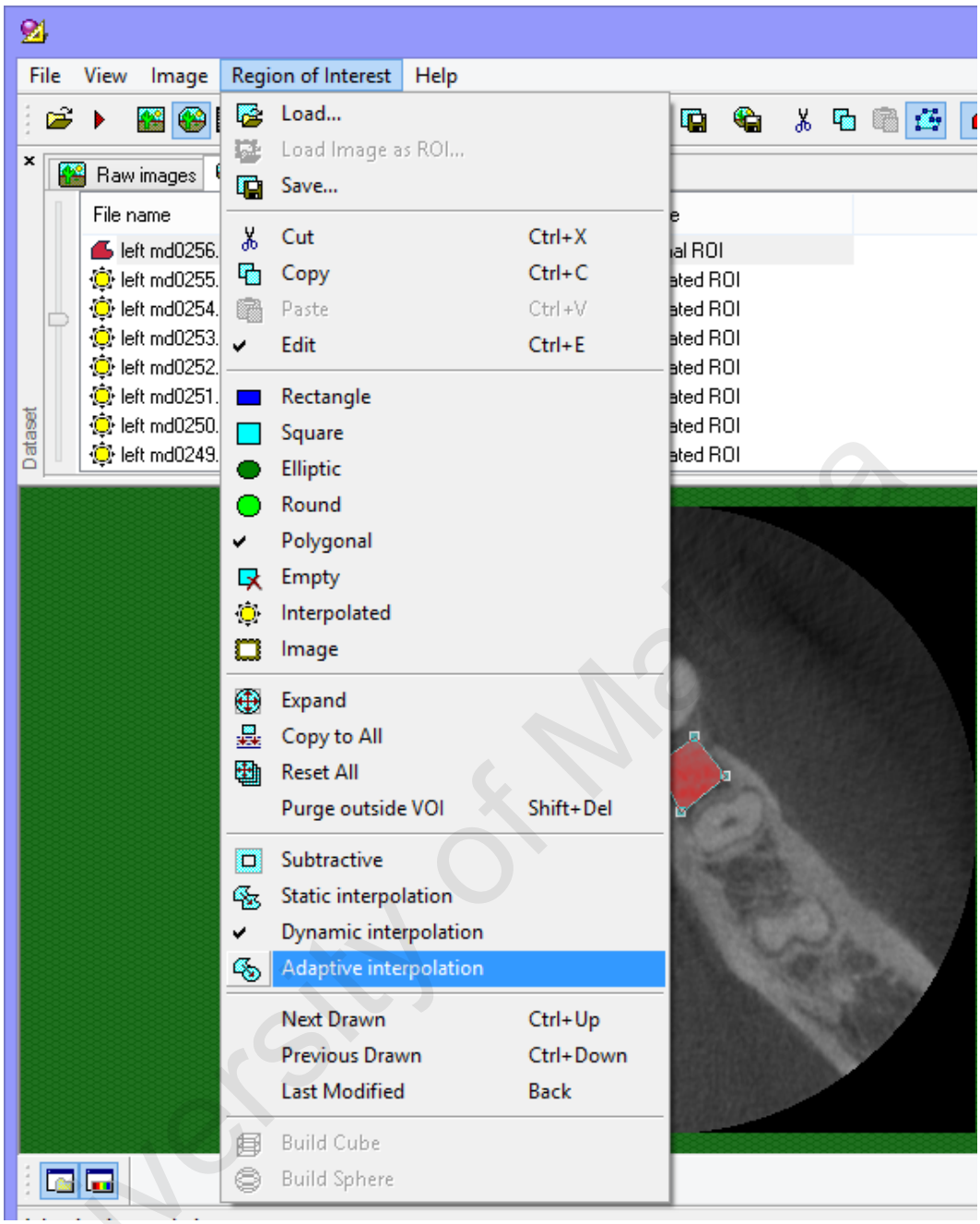

Figure 3.16 Applying Adaptive Interpolation to Select the Region of Interest

The correctly identified ROI was then transferred to the binary images tab (Figure 3.17) where white color representing the solid regions of the bony trabeculae areas that lie within the range of the binary threshold selection (Bruker, 2013). The spaces among the bony mandibular trabeculae appeared as black. The toggle tab was then applied to filter the image where only the region of interest remained black-and-white while the

image outside the region of interest was the same color as the background. Then, the histogram that represented the binary threshold was changed to “histogram/from selection tab”. This is to ensure that the histogram distribution of the brightness (solid areas of bone) was derived only from the previously selected layers instead of from the whole dataset. Hence, allowing the histogram to correctly represent the binary threshold of the selected ROI. Next the threshold values of the histogram were determined by visual evaluation where the selected threshold values allowed a complete visualisation of the buccal cortical plate of the mandible. The boundaries for the threshold value selection was set using 2 sliders (above and below the histogram window) (Figure 3.17). This selection of threshold value was only done for the control side. These values were then fixed as the standard threshold value for each patient. This allowed the comparison of the intervention side to the control side.

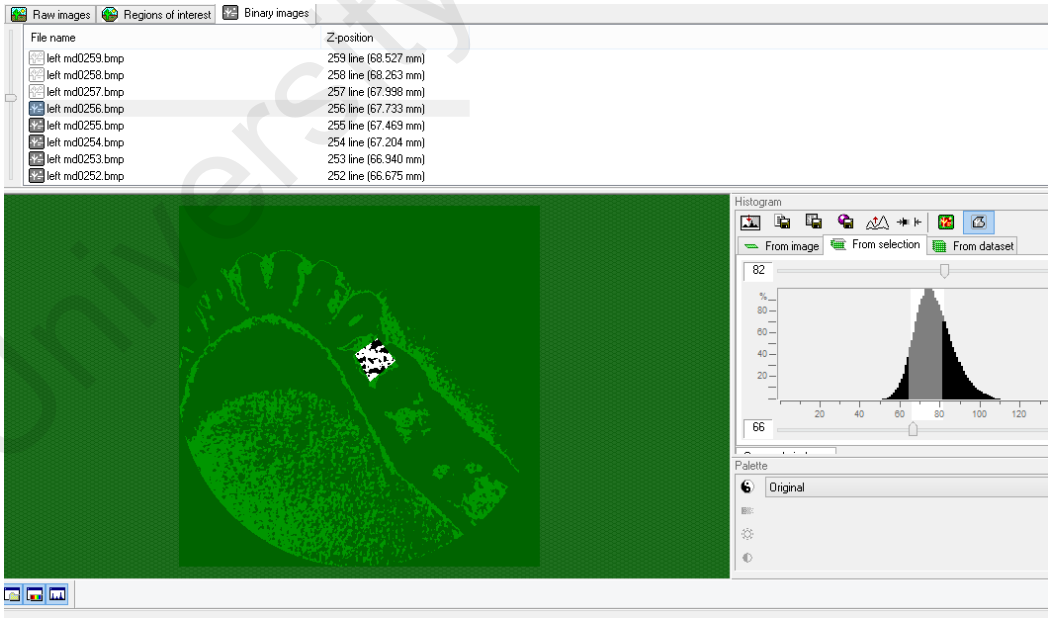

Figure 3.17 The binary image tab showing the region of interest in the binary image

After converting the selected region of interest (i.e. V1) into binary, the data was then transferred to the processed images tab (Figure 3.18) and then a 3D analysis was

generated which would provide the percentage of bone volume within the region of interest which represented the trabecular bone volume fraction (BV/TV) (Figure 3.19 – 3.20).

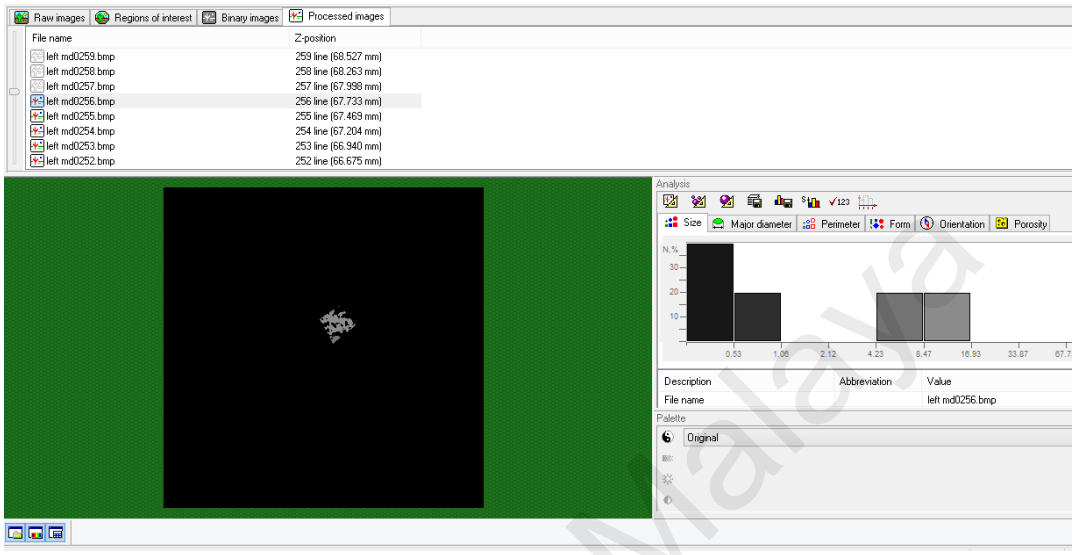

Figure 3.18 Processed Images Tab

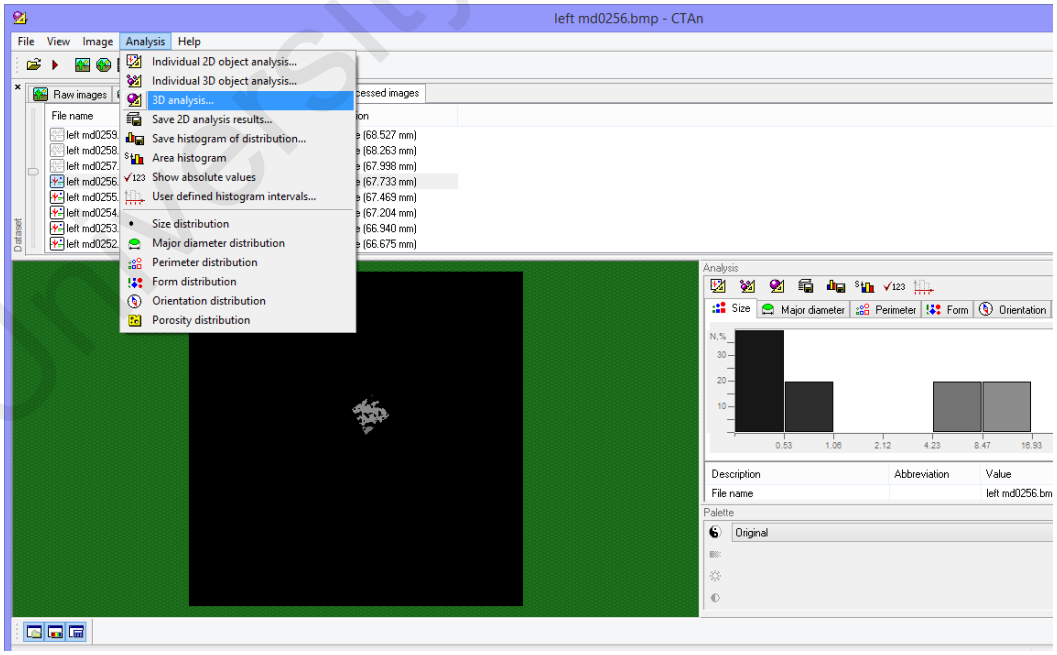

Figure 3.19 Tab Showing the 3D Analysis Selection

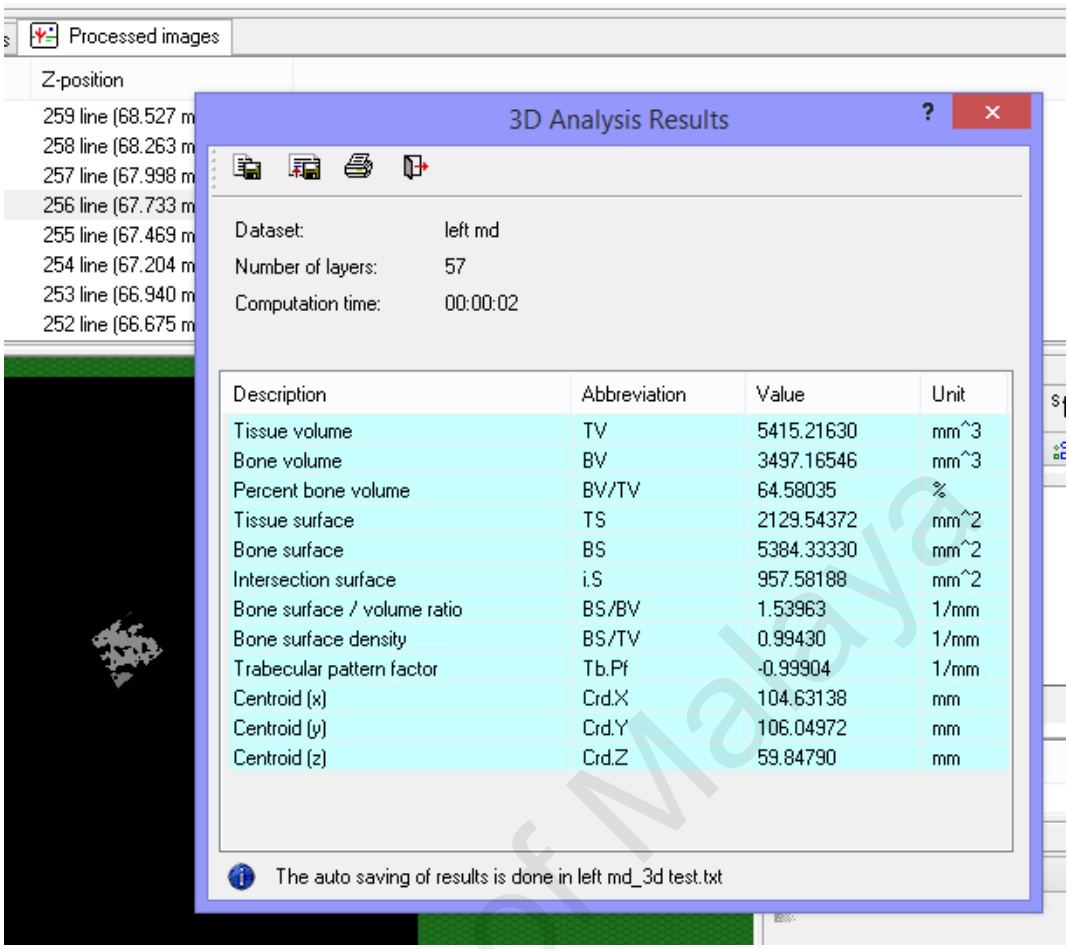

Figure 3.20 3D Analysis Result Showing BV/TV Values That Represent Trabecular Bone Volume Fraction

This process was then repeated to obtain the BV/TV values for all ROIs (V1, V2, V3, H1, H2 and H3) for each individual patient. The data was then recorded for each individual patient and then collected in a general database.

3.8 Calibration of examiners

Calibration of the digital callipers was done every time prior to clinical measurement of canine movement. Intra-observer and inter-observer values for clinical measurement of canine retraction were assessed between examiner SS and examiner WMC.

Examiner TNHK was trained by Norliza Ibrahim (Ibrahim et al., 2014) to use the CT Analyser software for the purpose of measuring trabecular bone volumetric fraction. A period of one month was allocated for familiarization of technique and software. Following that, five random samples were selected and their BV/TV values obtained for statistical determination of intra-observer reliability. This was repeated two weeks later and the intra-observer reliability calculated. In conjunction with that, this set of results were also compared against the result of another experienced researcher (M) for analysis of inter-observer reliability.

CHAPTER 4: RESULTS

4.1 Instrument Validation

4.1.1 Calibration of Examiners for Clinical Measurement of Rate of Canine Retraction

Intra-observer values were excellent for both examiners SS and WMC (ICC 0.94 and 0.91 respectively). Their inter-examiner reliability score was also excellent at ICC 0.93.

4.1.2 Calibration of Examiners for Radiographic Measurement of Bone Volumetric Fraction (BV/TV)

Examiner TNHK was trained by Norliza Ibrahim (Ibrahim et al., 2014) to measure trabecular bone volumetric fraction using The CT Analyser program. After one month of familiarization with the technique and use of the program, BV/TV values of five random samples were obtained and repeated 2 weeks later for intra-observer reliability (Table 4.1). Comparison was done against the result of another experienced researcher (M) for analysis of inter-observer reliability.

Table 4.1 BV/TV values used for intra- and inter-observer reliability tests

Sample	BV/TV (%) observer		
	TNHK		M
	1st measurement	2nd measurement	
1	72.22	81.02	73.41
2	58.96	57.44	49.62
3	42.41	44.92	52.60
4	60.75	60.04	62.47
5	50.93	55.25	45.80

4.1.3 Error of Method

In 1954, Fisher introduced a modified Pearson correlation coefficient as Intraclass correlation coefficient (ICC) (Fisher, 1954). In research, the intraclass correlation coefficient is used to evaluate inter-rater and intra-rater reliability analyses (Koo & Li, 2016). According to Shrout and Fleiss, a 2-way mixed-effect model and an "absolute agreement" type should be used to test intra-rater reliability with multiple scores from the same rater (Shrout & Fleiss, 1979). Hence for this study, ICC estimates and their 95% confident intervals were calculated using SPSS statistical package version 22 based on a mean-rating (k = 2), absolute-agreement, 2-way mixed-effects model. Both intra-rater (0.96) and inter-rater (0.90) ICC scores indicated excellent and good reliability respectively (Tables 4.2 and 4.3).

Table 4.2 Intra Examiner, ICC 0.96

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.931[a]	.558	.992	33.538	4	4	.002
Average Measures	.964[c]	.716	.996	33.538	4	4	.002

Table 4.3 Inter Examiner, ICC 0.90

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.814[a]	-.113	.979	8.011	4	4	.034
Average Measures	.897[c]	-.255	.990	8.011	4	4	.034

4.2 Workflow of the Research Project

For the clinical stage of this study, a total of 179 patients were assessed for eligibility. 147 participants did not meet the inclusion criteria and were thus excluded from the study. Two patients opted not to enter the trial for personal reasons. All 149 patients who were not entered into the trial continued to receive orthodontic treatment at the Faculty of Dentistry, University of Malaya. The 30 patients that fulfilled the inclusion and exclusion criteria were then randomized into three groups of different intervention intervals. The first patient was entered into the trial in September 2014 and the data collection for the clinical part was completed in February 2017. There were no dropouts at the clinical stage of the trial.

For the radiographic stage of the trial, there were six dropouts, two from the 4 weekly MOP interval (Group 1) and four from the 8 weekly MOP interval (Group 2) (Figure 4.1).

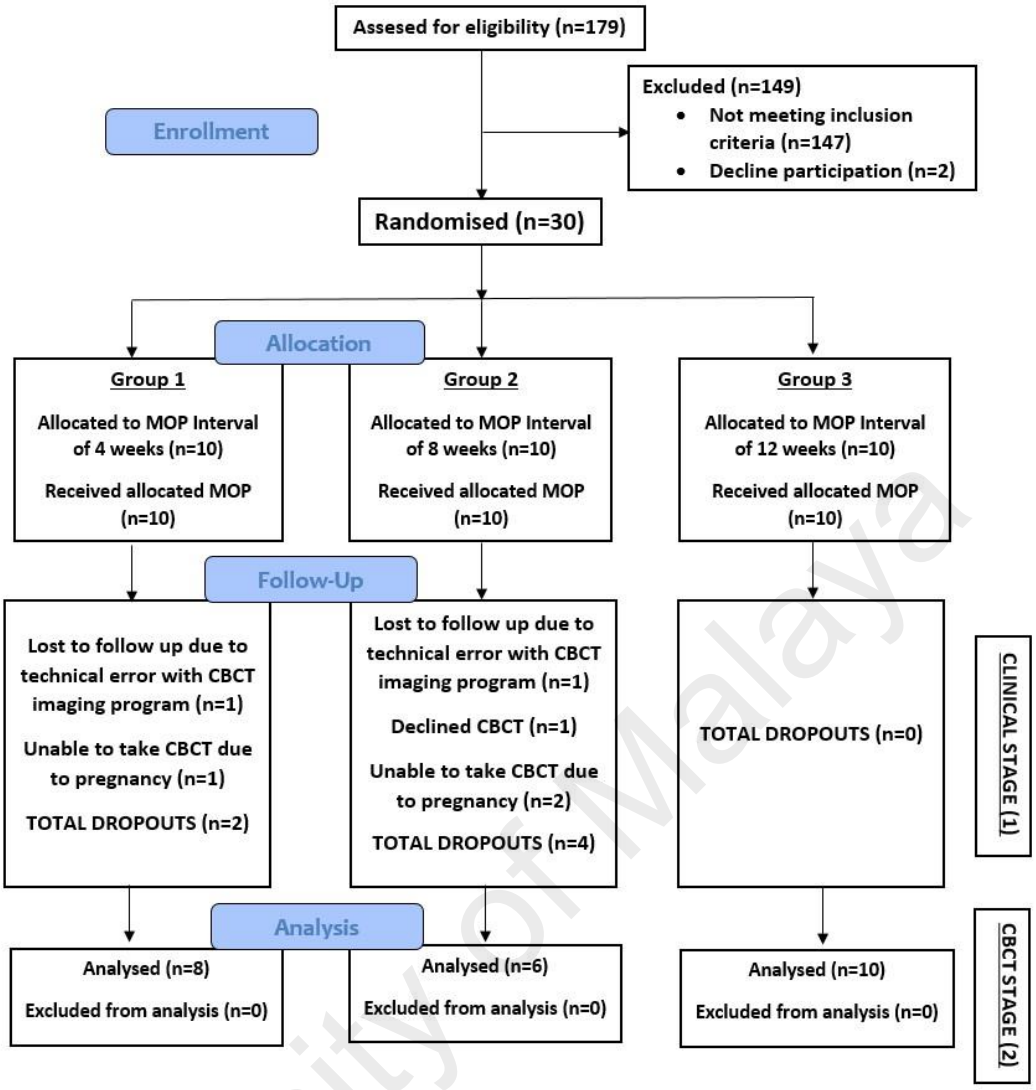

Figure 4.1 Consort Flow Diagram Depicting the Workflow of the Trial

4.3 Demographic Results

A total of 30 subjects were recruited for the first stage of this trial. They were randomly distributed into three groups of 10 subjects each, based on the MOP intervention intervals of 4 weeks (Group 1), 8 weeks (Group 2) and 12 weeks (Group 3). CBCT data was available only for 24 out of the 30 subjects in the second stage of this trial.

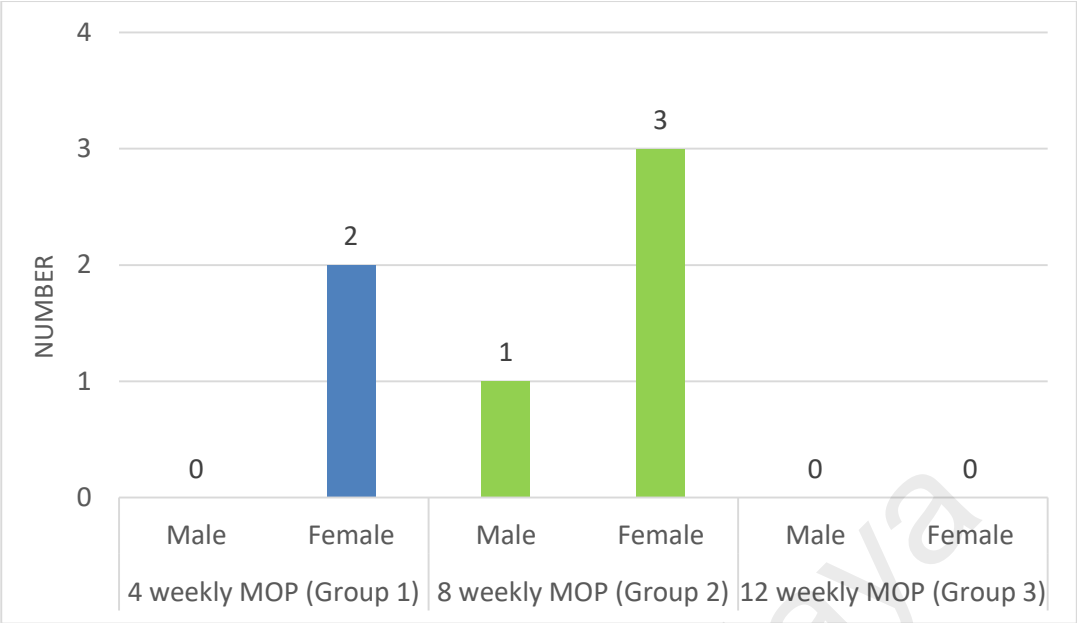

Figure 4.2 Demographics for Dropouts Based On MOP Intervals

The reasons for dropouts were due to pregnancy at the time for CBCT imaging (n=3), technical error with CBCT imaging program (n=2) and patient refusal for CBCT imaging (n=1) (Figure 4.1). The final number of subjects for CBCT analysis were 8 subjects for the 4 weekly MOP interval group, 6 for the 8 weekly group and 10 for the 12 weekly group (Figure 4.3).

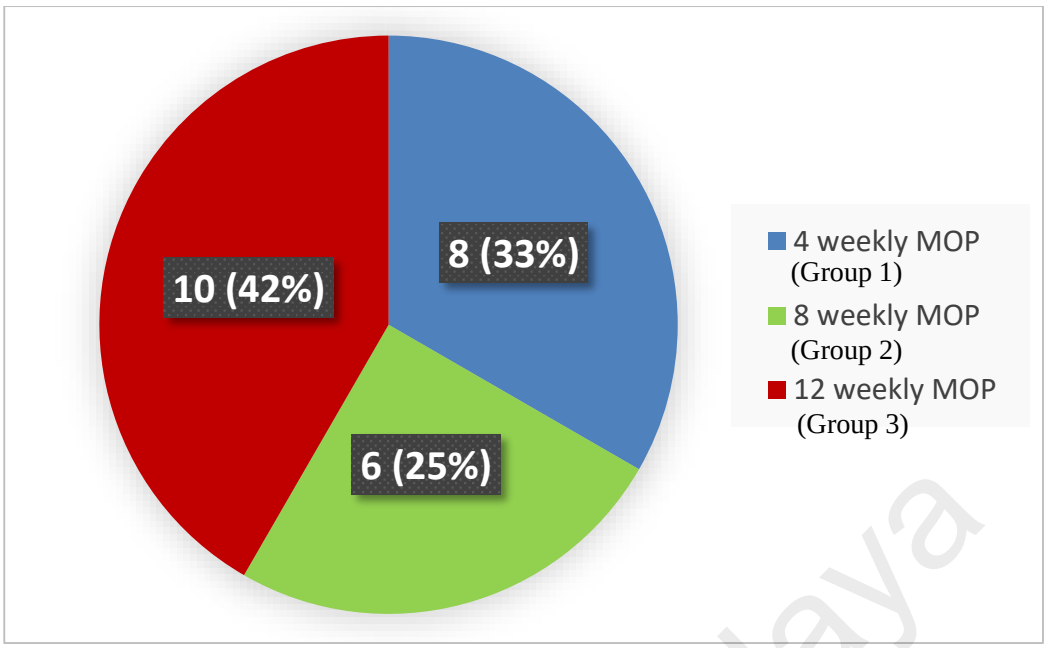

Figure 4.3 Proportion of Group Subjects

The subjects' age ranged from 19 to 27-years-old, with the mean ages of 23.4 ± 1.8 years for the 4 weekly MOP interval group, 24.0 ± 2.6 years for the 8 weekly group and 23.3 ± 2.8 years for the 12 weekly group (Figure 4.4). The difference between the groups were not significant (p=0.85 calculated from ANOVA).

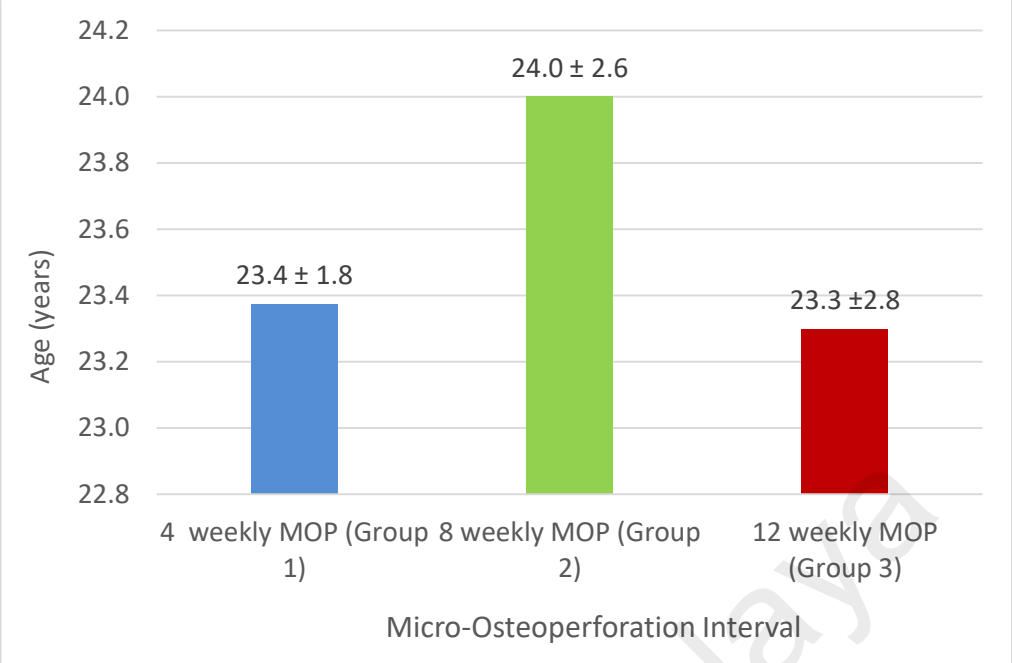

Figure 4.4 Demographics for Age Based on MOP Intervals

(ANOVA mean age between groups p=0.85)

A total of 6 male (25%) and 18 female (75%) patients participated in the study. The gender distribution according to MOP intervals is reported in Figure 4.5. There was no statistically significant association between gender and MOP interval grouping (p=0.64, calculated by Pearson Chi-Square).

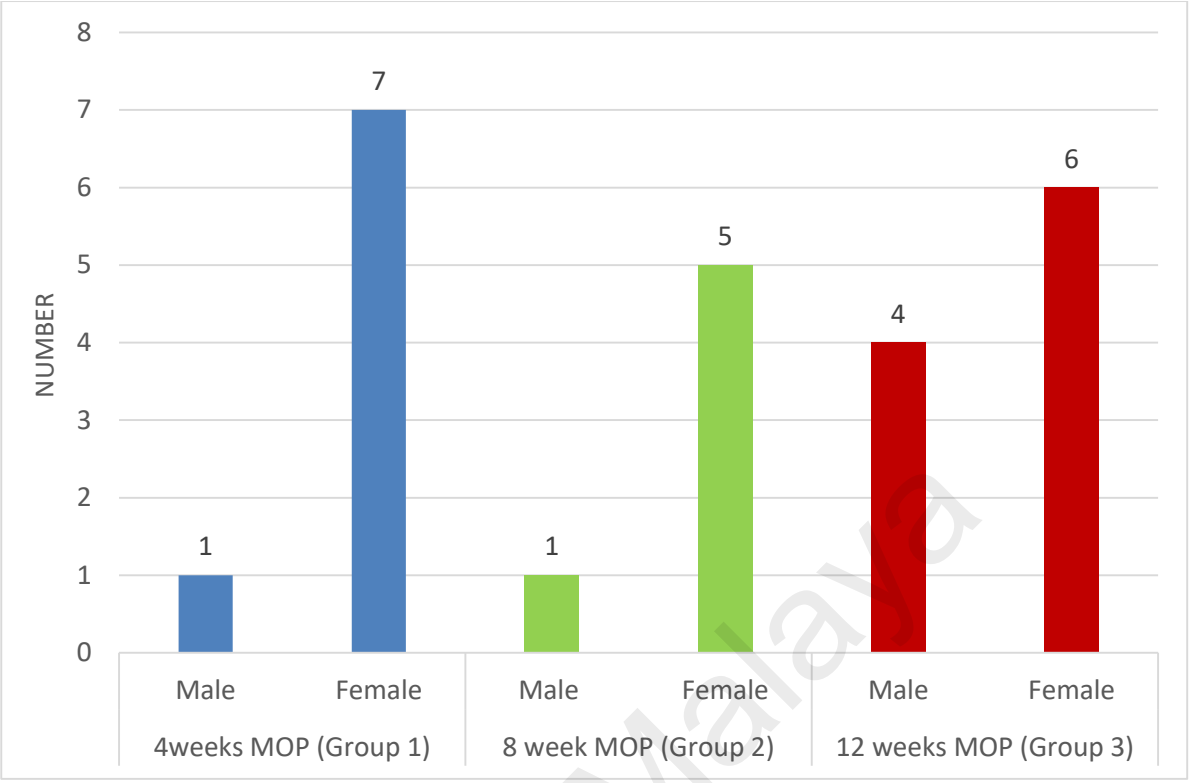

Figure 4.5 Participant Demographics for Gender Based on MOP Intervals

(Pearson Chi-Square for association between gender and intervention groups, p=0.64)

4.4 Normality of Data

The Shapiro-Wilk Test was chosen to test the data for normality spread as it was more appropriate for small sample sizes (< 50 samples) and had better power detection as compared to Kolmogorov-Smirnov test (Ghasemi & Zahediasl, 2012). Data for all groups showed significant values of more than 0.05 which indicated that the data in these groups were normally distributed and could be analysed with parametric statistical tests (Table 4.4).

Paired t-tests were used to analyse the means between two groups in a split-mouth design (Pandis, 2015) while ANOVA test was used to analyse means of more than two groups.

Table 4.4 Normality Spread of Data

Section	Side	Shapiro-Wilk	
		Statistic	Significance
V1	Control	0.96	0.49
	Intervention	0.96	0.37
V2	Control	0.95	0.27
	Intervention	0.97	0.61
V3	Control	0.93	0.08
	Intervention	0.86	0.43
H1	Control	0.98	0.78
	Intervention	0.94	0.20
H2	Control	0.93	0.08
	Intervention	0.96	0.50
H3	Control	0.92	0.05
	Intervention	0.95	0.24

(* $p<0.05$ is significant)

4.5 Vertical and horizontal distribution of mandibular trabeculae pattern between control and intervention side

Comparison was carried out for the BV/TV and canine movement rates between control and intervention sides. Mandibular trabecular bone volumetric fraction (BV/TV) was significantly lesser (p=0.00) in the MOP intervention site as compared to the contralateral control site, specifically V1 section (interdental bone between mandibular canine and mandibular second premolar, the area of active orthodontic tooth movement during canine retraction) (Table 4.6). Similarly, mandibular trabecular BV/TV was also significantly reduced at H2 section (Table 4.6), which overlapped with V1 section. The BV/TV at both V1 and H2 sections was approximately 10% less than the control sites. The other sections of V2, H1 and H3 showed less mandibular BV/TV when compared to the control side but this was not statistically significant (Table 4.6). Similarly, the higher BV/TV at the intervention section of V3 was not statistically significant (Table 4.6). On

average, the canine movement rate at the MOP intervention sites was significantly faster than at the control sites by 1.25mm/4 months or approximately 0.31mm/month.

Table 4.5 Mean values of Bone Volumetric Fraction (BV/TV) and Canine Movement Rates for Control and Intervention sides

Measured Variable	Side	N	Mean	Standard Deviation	Std. Error Mean
BV/TV – V1 (%)	Control	24	63.97	7.64	1.56
	Intervention	24	54.18	14.70	3.00
BV/TV – V2 (%)	Control	24	63.24	12.14	2.48
	Intervention	24	58.04	13.48	2.75
BV/TV – V3 (%)	Control	24	62.98	13.01	2.65
	Intervention	24	64.57	10.87	2.21
BV/TV – H1 (%)	Control	24	49.27	23.75	4.85
	Intervention	24	41.74	28.33	5.78
BV/TV – H2 (%)	Control	24	61.65	8.79	1.80
	Intervention	24	51.63	15.25	3.11
BV/TV – H3 (%)	Control	24	97.54	15.03	3.07
	Intervention	24	67.46	15.83	3.23
Canine movement rate (mm/4months)	Control	24	2.77	1.33	0.27
	Intervention	24	4.03	1.53	0.31

Table 4.6 Paired Sample Test Statistics of BV/TV and Canine Movement Rates for Intervention as Compared to Control Sides

Measured Variable	Paired difference between control and intervention sides				Significance (2-tailed)
	Mean	Standard Deviation	95% confidence interval of the difference		
			Lower	Upper	
BV/TV – V1 (%)	9.79	11.89	4.77	14.81	0.00*
BV/TV – V2 (%)	5.20	13.19	-0.37	10.77	0.07
BV/TV – V3 (%)	-1.59	10.47	-6.01	2.83	0.47
BV/TV – H1 (%)	7.53	25.34	-3.17	18.23	0.16
BV/TV – H2 (%)	10.03	13.41	4.36	15.69	0.00*
BV/TV – H3 (%)	0.08	12.06	-5.01	5.17	0.97
Canine movement rate (mm/4 months)	-1.25	0.79	-1.59	-0.92	0.00*

(* $p<0.05$ is significant, positive values indicate control side value is larger than intervention side, negative value indicates intervention side value is larger than control side)

In analysing the distribution of BV/TV vertically across the alveolar bone, the BV/TV at the control side stayed almost the same in the direction towards the apical, the difference in between the vertical levels being not statistically significant (Table 4.7 and Figure 4.6). However horizontally, the BV/TV at section H1 (interdental bone between lateral incisor and canine), H2 (between canine and second premolar) and H3 (between second premolar and molar) is significantly different (p=0.00) with the BV/TV increasing in the direction towards the posterior (Table 4.7 and Figure 4.7).

At the intervention side, the BV/TV value increased in the direction towards the apical, the difference in BV/TV between the vertical levels being statistically significant (p=0.03) (Table 4.7 and Figure 4.6). Horizontally, the BV/TV is significantly different (p=0.00) between H1, H2 and H3 with the BV/TV increasing in the direction towards the posterior, similar to the horizontal distribution at the control side (Table 4.7 and Figure 4.7).

Table 4.7 Distribution of Bone Volumetric Fraction across alveolar bone

Side	Section	Mean (%)	Std. Deviation	Significance (analysed by ANOVA)
Control	V1	63.97	7.63	
	V2	63.24	12.14	0.95
	V3	62.98	13.01	
	H1	49.27	23.75	
	H2	61.65	8.79	0.00*
	H3	67.54	15.03	
Intervention	V1	54.18	14.70	
	V2	58.04	13.48	0.03*
	V3	64.57	10.87	
	H1	41.74	28.33	
	H2	51.62	15.25	0.00*
	H3	67.46	15.83	

(* p<0.05 is significant)

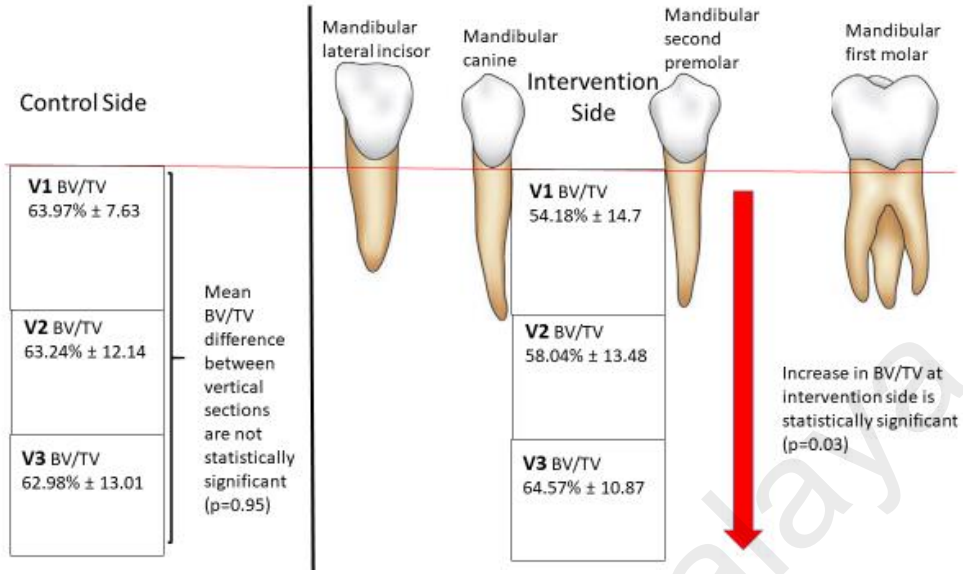

Figure 4.6 Schematic Diagram Showing Vertical Distribution of Bone Volumetric Fraction for Control and Intervention Sides

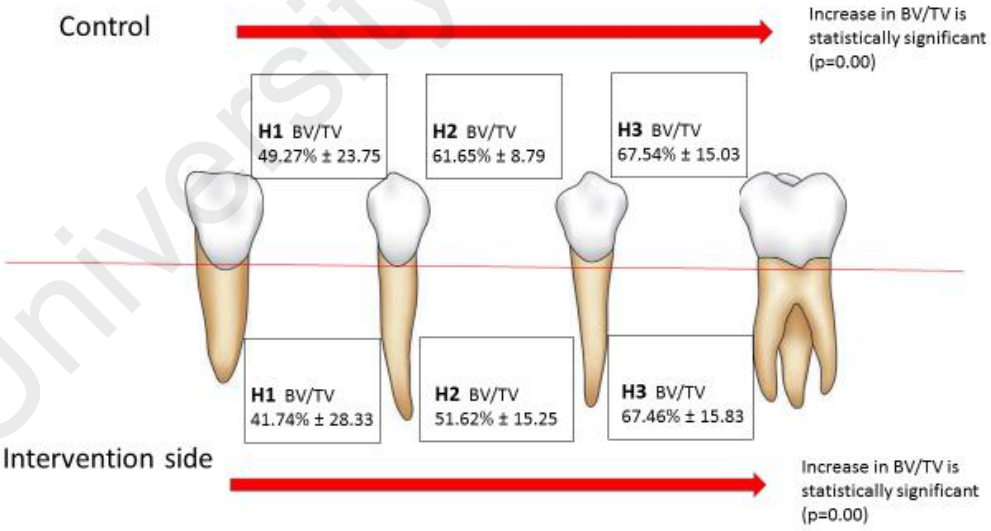

Figure 4.7 Schematic Diagram Showing Horizontal Distribution of Bone Volumetric Fraction for Control and Intervention Sides

4.6 Distribution of vertical and horizontal mandibular trabeculae pattern according to different micro-osteoperforation intervals

4.6.1 Micro-osteoperforation at 4 weekly interval (Group 1)

Data analysis based on MOP intervention intervals showed that with the 4 weekly MOP intervention, BV/TV at the V1 and H2 sections was significantly reduced (p=0.01 and p=0.04 respectively, Table 4.9) by almost 15%, both sites representing the interdental bone between mandibular canine and second premolar which was also the area of active orthodontic tooth movement during canine retraction, as compared to the control side. The mandibular BV/TV in the other sections namely V2, V3 and H1 were lesser when compared to the control side but this was not statistically significant. The mandibular BV/TV at H3 was almost similar to the control side (Table 4.9).

The canine movement rate was significantly faster (p=0.00) at the intervention than at the control side by 1.49mm over the 4 month period.

With MOP done at 4 weekly interval, the BV/TV at the intervention side was much higher in the direction towards the apical, the difference between the intervention vertical levels being significantly different (p=0.01, Table 4.10). Horizontally, the BV/TV increased in the direction towards posterior (p=0.01, Table 4.10).

Table 4.8 Mean Values of Bone Volumetric Fraction and Canine Movement Rates for Control and 4 Weekly MOP Intervention Interval

Measured Variable	Side	n	Mean	Standard Deviation	Std. Error Mean
Pair 1 BV/TV (%)	Control	8	59.15	5.28	1.87
	Intervention V1	8	44.42	15.47	5.47
Pair 2 BV/TV (%)	Control	8	65.07	10.49	3.71
	Intervention V2	8	59.13	14.09	4.98
Pair 3 BV/TV (%)	Control	8	67.88	13.95	4.93
	Intervention V3	8	67.38	11.24	3.97
Pair 4 BV/TV (%)	Control	8	38.27	22.65	8.01
	Intervention H1	8	34.44	18.30	6.47
Pair 5 BV/TV (%)	Control	8	58.33	7.09	2.51
	Intervention H2	8	43.25	18.84	6.66
Pair 6 BV/TV (%)	Control	8	65.37	18.89	6.68
	Intervention H3	8	65.43	18.26	6.46
Pair 7 Canine movement rate (mm/4months)	Control	8	3.08	1.24	0.44
	Intervention	8	4.57	1.74	0.62

Table 4.9 Paired Sample Test Statistics of Bone Volumetric Fraction and Canine Movement Rates for 4 weekly MOP Intervention Interval as Compared to Control Sides

Measured Variable	Paired difference				Significance (2-tailed)
	Mean	Standard Deviation	95% confidence interval of the difference		
			Lower	Upper	
Pair 1 BV/TV Control V1 – Intervention V1 (%)	14.73	12.88	3.96	25.50	0.01*
Pair 2 BV/TV Control V2 – Intervention V2 (%)	5.95	18.22	-9.29	21.18	0.39
Pair 3 BV/TV Control V3 – Intervention V3 (%)	0.50	13.41	-10.71	11.71	0.92
Pair 4 BV/TV Control H1 – Intervention H1 (%)	3.82	26.99	-18.74	26.39	0.70
Pair 5 BV/TV Control H2 – Intervention H2 (%)	15.08	17.86	0.16	30.01	0.04*
Pair 6 BV/TV Control H3 – Intervention H3 (%)	-0.06	16.38	-13.75	13.63	0.99
Pair 7 Control Canine movement rate – Intervention Canine movement rate (mm/4 months)	-1.49	0.78	-2.14	-0.84	0.00*

(* p<0.05 is significant, positive values indicate control side value is larger than intervention side, negative value indicates intervention side value is larger than control side)

Table 4.10 Distribution of Bone Volumetric Fraction with 4 Weekly MOP Interval across Alveolar Bone

Side	Section	Mean (%)	Std. Deviation	Significance (analysed by ANOVA)
Intervention	V1	44.42	15.47	
	V2	59.13	14.09	0.01*
	V3	67.38	11.24	
	H1	34.44	18.30	
	H2	43.25	18.84	0.01*
	H3	65.43	18.26	

(* *p*<0.05 is significant)

4.6.2 Micro-osteoperforation at 8 Weekly Interval (Group 2)

At the 8 weekly MOP intervention interval, no significant difference was found between intervention and control sites. The mandibular BV/TV was insignificantly lesser at the V1, V2, H1 and H2, and insignificantly more at V3 and H3 intervention sections (Table 4.12). At the MOP intervention side, canine retraction rate was significantly faster by 1.12mm/4 months than at the control side (Table 4.12).

The vertical and horizontal distribution differences in BV/TV were found to be statistically not significant. At the intervention side, it is insignificantly lesser at V2 but insignificantly more at V3. Horizontally, the BV/TV of the intervention side increased insignificantly towards the posterior (Table 4.13).

Table 4.11 Mean Values of Bone Volumetric Fraction and Canine Movement Rates for Control and 8 Weekly MOP Intervention Interval

Measured Variable	Side	n	Mean	Standard Deviation	Std. Error Mean
Pair 1 BV/TV (%)	Control	6	66.67	9.21	3.76
	Intervention V1	6	59.66	11.69	4.77
Pair 2 BV/TV (%)	Control	6	62.61	15.43	6.30
	Intervention V2	6	55.10	15.59	6.36
Pair 3 BV/TV (%)	Control	6	60.68	9.92	4.05
	Intervention V3	6	61.71	13.96	5.70
Pair 4 BV/TV (%)	Control	6	48.86	22.72	9.28
	Intervention H1	6	40.84	28.61	11.68
Pair 5 BV/TV (%)	Control	6	61.71	11.57	4.72
	Intervention H2	6	55.51	10.29	4.20
Pair 6 BV/TV (%)	Control	6	70.38	14.29	5.83
	Intervention H3	6	71.70	19.06	7.78
Pair 7 Canine movement rate (mm/4months)	Control	6	1.94	0.92	0.37
	Intervention	6	3.06	1.34	0.55

Table 4.12 Paired Sample Test Statistics of Bone Volumetric Fraction and Canine Movement Rates for 8 Weekly MOP Intervention Interval as Compared to Control Sides

Measured Variable	Paired difference				Significance (2-tailed)
	Mean	Standard Deviation	95% confidence interval of the difference		
			Lower	Upper	
Pair 1 BV/TV Control V1 – Intervention V1 (%)	7.01	9.73	-3.20	17.22	0.14
Pair 2 BV/TV Control V2 – Intervention V2 (%)	7.51	8.71	-1.63	16.65	0.09
Pair 3 BV/TV Control V3 – Intervention V3 (%)	-1.03	9.70	-11.22	9.15	0.80
Pair 4 BV/TV Control H1 – Intervention H1 (%)	8.02	21.55	-14.60	30.64	0.40
Pair 5 BV/TV Control H2 – Intervention H2 (%)	6.19	12.69	-7.12	19.51	0.29
Pair 6 BV/TV Control H3 – Intervention H3 (%)	-1.31	6.67	-8.31	5.68	0.65
Pair 7 Control Canine movement rate – Intervention Canine movement rate (mm/4months)	-1.12	0.60	-1.75	-0.48	0.01*

(* p<0.05 is significant, positive values indicate control side value is larger than intervention side, negative value indicates intervention side value is larger than control side)

Table 4.13 Distribution of Bone Volumetric Fraction with 8 Weekly MOP Interval across Alveolar Bone

Side	Section	Mean (%)	Std. Deviation	Significance (analysed by ANOVA)
Intervention	V1	59.66	11.69	
	V2	55.10	15.59	0.70
	V3	61.71	13.96	
	H1	40.84	28.61	
	H2	55.51	10.29	0.06
	H3	71.70	19.06	

(* *p*<0.05 is significant)

4.6.3 Micro-osteoperforation at 12 Weekly Interval (Group 3)

When MOP intervention was done at 12 weekly interval, there was a significant (p=0.02) reduction of 8% in mandibular BV/TV at the H2 section of the intervention side, as compared to control side. The BV/TV was insignificantly lesser at the V1, V2, H1 and H3 intervention sections (Table 4.15). At the V3 section, there was an insignificantly higher BV/TV at the intervention than at the control site (Table 4.15). Canine movement was at a higher rate at the intervention side by about 1.14mm/4 months, as compared to the control side (Table 4.15).

Within the 12 weekly MOP interval group, the vertical and horizontal distribution differences in BV/TV were found to be statistically not significant (Table 4.16). Vertically, the BV/TV increased insignificantly towards the apical at the intervention side (Table 4.16). Horizontally, the BV/TV increased insignificantly towards the posterior (Table 4.16).

Table 4.14 Mean Values of Bone Volumetric Fraction and Canine Movement Rates for Control and 12 Weekly MOP Intervention Interval

Measured Variable	Side	n	Mean	Standard Deviation	Std. Error Mean
Pair 1 BV/TV (%)	Control	10	66.22	7.00	2.21
	Intervention V1	10	58.70	12.77	4.04
Pair 2 BV/TV (%)	Control	10	62.16	12.42	3.93
	Intervention V2	10	58.94	12.93	4.09
Pair 3 BV/TV (%)	Control	10	60.45	13.95	4.41
	Intervention V3	10	64.04	9.08	2.87
Pair 4 BV/TV (%)	Control	10	58.31	23.60	7.46
	Intervention H1	10	48.12	35.25	11.15
Pair 5 BV/TV (%)	Control	10	64.27	8.17	2.58
	Intervention H2	10	56.00	12.86	4.07
Pair 6 BV/TV (%)	Control	10	67.57	13.29	4.20
	Intervention H3	10	66.54	12.76	4.04
Pair 7 Canine movement rate (mm/4months)	Control	10	3.03	1.50	0.47
	Intervention	10	4.17	1.31	0.42

Table 4.15 Paired Sample Test Statistics of Bone Volumetric Fraction and Canine Movement Rates for 12 weekly MOP Intervention Interval as Compared to Control Sides

Measured Variable	Paired difference				Significance (2-tailed)
	Mean	Std. Deviation	95% confidence interval of the difference		
			Lower	Upper	
Pair 1 BV/TV Control V1 – Intervention V1 (%)	7.51	12.12	-1.16	16.19	0.08
Pair 2 BV/TV Control V2 – Intervention V2 (%)	3.22	11.67	-5.13	11.56	0.41
Pair 3 BV/TV Control V3 – Intervention V3 (%)	-3.59	8.92	-9.97	2.79	0.24
Pair 4 BV/TV Control H1 – Intervention H1 (%)	10.20	28.21	-9.99	30.38	0.28
Pair 5 BV/TV Control H2 – Intervention H2 (%)	8.28	9.29	1.63	14.92	0.02*
Pair 6 BV/TV Control H3 – Intervention H3 (%)	1.03	11.66	-7.31	9.37	0.79
Pair 7 Control Canine movement rate – Intervention Canine movement rate (mm/4months)	-1.14	0.93	-1.81	-0.48	0.00*

(* p<0.05 is significant, positive values indicate control side value is larger than intervention side, negative value indicates intervention side value is larger than control side)

Table 4.16 Distribution of Bone Volumetric Fraction with 12 Weekly MOP Interval across Alveolar Bone

Side	Section	Mean (%)	Std. Deviation	Significance (analysed by ANOVA)
Intervention	V1	58.70	12.77	
	V2	58.94	12.93	0.53
	V3	64.04	9.08	
	H1	48.12	35.25	
	H2	56.00	12.86	0.21
	H3	66.54	12.76	

(* p<0.05 is significant)

4.6.4 Comparison between intervention intervals

Data analysis on the frequency of intervention intervals showed that only the mean BV/TV differences at V1 and H2 sections of 4 weekly, and section H2 of 12 weekly intervals were statistically significant (Table 4.17).

Table 4.17 Paired Sample Test Statistics of BV/TV and Canine Movement Rates for the Intervention as Compared to Control Sites, according to MOP Intervals

Measured Variable	Paired difference for different intervention intervals as compared to control sites								
	4 weekly MOP Interval			8 weekly MOP Interval			12 weekly MOP Interval		
	Mean	SD	Sig	Mean	SD	Sig	Mean	SD	Sig
BV/TV – V1 (%)	14.73*	12.88	0.01	7.01	9.73	0.14	7.51	12.12	0.08
BV/TV – V2 (%)	5.95	18.22	0.39	7.51	8.71	0.08	3.22	11.67	0.41
BV/TV – V3 (%)	0.50	13.41	0.92	-1.03	9.70	0.81	-3.59	8.92	0.24
BV/TV – H1 (%)	3.82	26.99	0.70	8.02	21.55	0.40	10.20	28.21	0.28
BV/TV – H2 (%)	15.08*	17.86	0.04	6.19	12.69	0.29	8.28*	9.29	0.02
BV/TV – H3 (%)	-0.06	16.38	0.99	-1.31	6.67	0.65	1.03	11.66	0.79
Canine movement rate (mm/4 months)	-1.49*	0.78	0.00	-1.12*	0.60	0.01	-1.14*	0.93	0.00

(* *p* value is significant at <0.05, positive values indicate control side value is larger than intervention side, negative value indicates intervention side value is larger than control side)

The differences in the horizontal and vertical BV/TV distribution were only significant for the four weekly frequency interval (Table 4.18). The horizontal distribution of BV/TV increased significantly towards the posterior at both control and intervention sides, whilst

the BV/TV increased significantly towards the apical of the intervention side, with statistical significance found between vertical sections.

Table 4.18 Vertical and Horizontal Distribution of BV/TV across Alveolar Bone According to Different MOP Intervals

Intervention	4 weekly MOP Interval			8 weekly MOP Interval			12 weekly MOP Interval		
	Mean (%)	SD	P value	Mean (%)	SD	P value	Mean (%)	SD	P value
V1	44.42	15.47		59.66	11.69		58.70	12.77	
V2	59.13	14.09	0.01*	55.10	15.59	0.70	58.94	12.93	0.53
V3	67.38	11.24		61.71	13.96		64.04	9.08	
H1	34.44	18.30		40.84	28.61		48.12	35.25	
H2	43.25	18.84	0.01*	55.51	10.29	0.06	56.00	12.86	0.21
H3	65.43	18.26		71.70	19.06		66.54	12.76	

(* *p* value for F test between means is significant at <0.05, analysed by ANOVA)

An ANOVA post hoc Tukey test was done for all 4 (Table 4.19), 8 (Table 4.20) and 12 (Table 4.21) weekly intervals to compare differences between each sections within each intervention side. The differences between sections were not statistically significant for all 8 and 12 weekly MOP intervals. BV/TV was only significantly different for the 4 weekly MOP interval, i.e. for horizontal and vertical sections of intervention side.

Only the differences between mean BV/TV of 4 weekly intervals and total sample population was statistically significant. However for canine retraction rate, the difference between mean of canine movement rate was statistically significant at all the intervention intervals.

Table 4.19 ANOVA with Post Hoc Tukey Test for Multiple Comparisons at 4 Weekly MOP Interval

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) level	(J) level	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
contolVertical	1	2	-5.92664750	5.26470050	.509	-19.1966956	7.3434006
		3	-8.72857750	5.26470050	.245	-21.9986256	4.5414706
	2	1	5.92664750	5.26470050	.509	-7.3434006	19.1966956
		3	-2.80193000	5.26470050	.856	-16.0719781	10.4681181
	3	1	8.72857750	5.26470050	.245	-4.5414706	21.9986256
		2	2.80193000	5.26470050	.856	-10.4681181	16.0719781
controlHorizontal	1	2	-20.06412	8.75620	.079	-42.1347	2.0065
		3	-27.10534*	8.75620	.015	-49.1760	-5.0347
	2	1	20.06412	8.75620	.079	-2.0065	42.1347
		3	-7.04122	8.75620	.705	-29.1118	15.0294
	3	1	27.10534*	8.75620	.015	5.0347	49.1760
		2	7.04122	8.75620	.705	-15.0294	29.1118
interventionVertical	1	2	-14.70808	6.85736	.105	-31.9925	2.5764
		3	-22.95689*	6.85736	.008	-40.2414	-5.6724
	2	1	14.70808	6.85736	.105	-2.5764	31.9925
		3	-8.24881	6.85736	.465	-25.5333	9.0357
	3	1	22.95689*	6.85736	.008	5.6724	40.2414
		2	8.24881	6.85736	.465	-9.0357	25.5333
interventionHorizontal	1	2	-8.80338	9.23554	.614	-32.0822	14.4755
		3	-30.98886*	9.23554	.008	-54.2677	-7.7100
	2	1	8.80338	9.23554	.614	-14.4755	32.0822
		3	-22.18548	9.23554	.064	-45.4643	1.0933
	3	1	30.98886*	9.23554	.008	7.7100	54.2677
		2	22.18548	9.23554	.064	-1.0933	45.4643

*. The mean difference is significant at the 0.05 level.

Table 4.20 ANOVA with Post Hoc Tukey Test for Multiple Comparisons at 8 Weekly MOP Interval

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) level	(J) level	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
controlVertical	1	2	4.05951667	6.84332098	.826	-13.7158060	21.8348393
		3	5.99422333	6.84332098	.663	-11.7810993	23.7695460
	2	1	-4.05951667	6.84332098	.826	-21.8348393	13.7158060
		3	1.93470667	6.84332098	.957	-15.8406160	19.7100293
	3	1	-5.99422333	6.84332098	.663	-23.7695460	11.7810993
		2	-1.93470667	6.84332098	.957	-19.7100293	15.8406160
controlHorizontal	1	2	-12.84391667	9.74273774	.407	-38.1503881	12.4625548
		3	-21.52260333	9.74273774	.102	-46.8290748	3.7838681
	2	1	12.84391667	9.74273774	.407	-12.4625548	38.1503881
		3	-8.67868667	9.74273774	.654	-33.9851581	16.6277848
	3	1	21.52260333	9.74273774	.102	-3.7838681	46.8290748
		2	8.67868667	9.74273774	.654	-16.6277848	33.9851581
interVertical	1	2	4.55861500	7.98919525	.838	-16.1930820	25.3103120
		3	-2.05071333	7.98919525	.964	-22.8024103	18.7009837
	2	1	-4.55861500	7.98919525	.838	-25.3103120	16.1930820
		3	-6.60932833	7.98919525	.692	-27.3610253	14.1423687
	3	1	2.05071333	7.98919525	.964	-18.7009837	22.8024103
		2	6.60932833	7.98919525	.692	-14.1423687	27.3610253
interHorizontal	1	2	-14.66778500	11.96340533	.457	-45.7423745	16.4068045
		3	-30.85462167	11.96340533	.052	-61.9292112	.2199678
	2	1	14.66778500	11.96340533	.457	-16.4068045	45.7423745
		3	-16.18683667	11.96340533	.389	-47.2614262	14.8877528
	3	1	30.85462167	11.96340533	.052	-.2199678	61.9292112
		2	16.18683667	11.96340533	.389	-14.8877528	47.2614262

Table 4.21 ANOVA with Post Hoc Tukey Test for Multiple Comparisons at 12 Weekly MOP Interval

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) levels	(J) levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
controlVertical	1	2	4.05933000	5.15090577	.713	-8.7119169	16.8305769
		3	5.76698200	5.15090577	.511	-7.0042649	18.5382289
	2	1	-4.05933000	5.15090577	.713	-16.8305769	8.7119169
		3	1.70765200	5.15090577	.941	-11.0635949	14.4788989
	3	1	-5.76698200	5.15090577	.511	-18.5382289	7.0042649
		2	-1.70765200	5.15090577	.941	-14.4788989	11.0635949
controlHorizontal	1	2	-5.96095500	7.30422633	.696	-24.0711830	12.1492730
		3	-9.25830900	7.30422633	.425	-27.3685370	8.8519190
	2	1	5.96095500	7.30422633	.696	-12.1492730	24.0711830
		3	-3.29735400	7.30422633	.894	-21.4075820	14.8128740
	3	1	9.25830900	7.30422633	.425	-8.8519190	27.3685370
		2	3.29735400	7.30422633	.894	-14.8128740	21.4075820
interVertical	1	2	-.23747600	5.24524004	.999	-13.2426169	12.7676649
		3	-5.33662700	5.24524004	.572	-18.3417679	7.6685139
	2	1	.23747600	5.24524004	.999	-12.7676649	13.2426169
		3	-5.09915100	5.24524004	.600	-18.1042919	7.9059899
	3	1	5.33662700	5.24524004	.572	-7.6685139	18.3417679
		2	5.09915100	5.24524004	.600	-7.9059899	18.1042919
interHorizontal	1	2	-7.87959600	10.23415630	.724	-33.2543442	17.4951522
		3	-18.42561700	10.23415630	.189	-43.8003652	6.9491312
	2	1	7.87959600	10.23415630	.724	-17.4951522	33.2543442
		3	-10.54602100	10.23415630	.564	-35.9207692	14.8287272
	3	1	18.42561700	10.23415630	.189	-6.9491312	43.8003652
		2	10.54602100	10.23415630	.564	-14.8287272	35.9207692

4.7 Correlation of the vertical and horizontal distribution of mandibular trabeculae pattern to the rate of canine tooth movement

As discussed above, only the differences between mean BV/TV of 4 weekly intervals and total sample population were statistically significant (p=0.01, p=0.00). However for canine retraction rate, the difference between mean of canine movement rate was statistically significant for all intervention intervals (Table 4.22).

Table 4.22 Mean Difference in Bone Volumetric Fraction at Section V1 and Rate of Canine Movement for Different Intervention Intervals

	Difference in mean BV/TV at V1 (%)		Difference in mean rate of canine movement (mm/4 months)	
	Mean	Std. Deviation	Mean	Std. Deviation
4 weekly MOP interval	14.73*	12.88	-1.49*	0.78
8 weekly MOP interval	7.01	9.73	-1.12*	0.68
12 weekly MOP interval	7.51	12.12	-1.14*	0.93
Combined all intervals	9.79*	11.89	-1.25*	0.79

(* $p<0.05$), positive values indicate control side value is larger than intervention side, negative value indicates intervention side value is larger than control side)

There was a negative value of Pearson correlation between the BV/TV of intervention site V1 and the rate of canine movement (Table 4.23). This meant that there is a statistically significant inverse linear relationship between BV/TV and rate of canine tooth movement at the intervention side. This indicated that as the BV/TV decreased due to MOP intervention, the rate of canine tooth movement increased. The strength of this association is moderate ($0.4 < \left| r \right| < 0.5$).

Table 4.23 Correlation between Bone Volumetric Fraction and Rate of Canine Movement

	Pearson Correlation (r)	2-tailed significance (*p<0.05)
Correlation of BV/TV Intervention V1 with Rate of Canine movement	-0.425	0.039*

CHAPTER 5: DISCUSSION

5.1 Demographic Data

5.1.1 Dropouts

Dropouts are defined as "the situation in which all outcome data are missing after a certain point" (Bell, Kenward, Fairclough, & Horton, 2013). These dropouts are frequently encountered during clinical trials and can present as a potential source of bias (Bell et al., 2013). According to Dr Rubin, there is a taxonomy of missingness which influences the selection of analyses to manage dropouts in a trial (Rubin, 1976). The data of patients who withdraw from a study for reasons unrelated to their disease or treatment are labelled "missing completely at random" because their absence from the trial are not related to the observed covariates which means that there are no systematic differences between dropouts and completers (Bell et al., 2013). Thus the mixed model based analyses can yield unbiased estimates of treatment effects. By using difference of mean based analyses, the mixed model analyses can on average, estimate unbiased treatment effect even if the dropout differs between treatment groups as the data from completers can be used to implicitly impute the missing values (Bell et al., 2013).

This study started with a sample size of 30 subjects at the clinical stage, which dwindled down to only 24 subjects in the radiographic stage, with a total dropout of 6 "missing completely at random" subjects due to a combination of pregnancy (n=3), technical error (n=2) and patient declination to CBCT imaging (n=1). Therefore in order to reduce bias, the data analyses was based around differences in mean of the control as compared to the intervention sides. Thus our split mouth study design was favourable where paired t–test analyses was the recommended analyses used (Pandis, 2015; Pandis, Walsh, Polychronopoulou, Katsaros, & Eliades, 2013). Although the overall total of 24 samples were within the required sample size of 24 subjects from the sample size calculation, the final number of subjects for the 8 weekly MOP interval group (n=6) fell

short of the minimum requirement of 8 subjects. Thus this study may be insufficiently powered to detect the treatment effects at 80% power for the 8 weekly MOP interval group. A previous study on the sample size of randomised clinical trials in orthodontic specialised journals suggested that a median of 46 subjects were required to show treatment effects at 80% power (Koletsi, Pandis, & Fleming, 2014). This limitation of our study is partly due to the fact that this trial was done in a university postgraduate clinic where time to conduct a clinical trial was very limited. Recommendations to improve future trials will be discussed in the limitations chapter.

5.1.2 Influence of Age on Bone Volumetric Patterns and Rate of Tooth Movement

There exists a relationship between age and bone volumetric fractions (BV/TV) despite the large variances in bone turnover rates (Khosla & Riggs, 2005; Raisz, 1999; Raisz & Seeman, 2001). Bone mass start to increase from birth and peaks around ages 15-20 years, after which a reduction in bone turnover rate leads to gradually reduced bone mass (Raisz & Seeman, 2001). Some of the age related bone loss changes include thinning of trabeculae and loss of trabecular connectivity (Rosen, Donahue, & Hunter, 1994) which reduces the bone volumetric fractions. Orthodontic tooth movement is also influenced by age, as there is greater tooth movement velocity in younger (<16 years old) than in older patients (>16 years old) (Dudic, Giannopoulou, & Kiliaridis, 2013). In addition, an animal study reported that surgical intervention to accelerate orthodontic tooth movement in growing rats are not as robust compared to adult rats (Librizzi et al., 2017). Hence, it is imperative that age be standardised to rule out any confounding factors, therefore one of our inclusion criteria was adult participants aged between eighteen and forty five years old. This resulted in the mean age of the study participants to be approximately twenty two to twenty four years of age. The age range was also well

distributed among the groups where the mean ages between the 3 intervention groups were almost similar.

5.1.3 Gender Distribution

Our study population had an unequal gender distribution, with 6 male and 18 female participants, reflecting the general orthodontic pattern as females were reported to be more likely to seek orthodontic treatment than males (Whitesides, Pajewski, Bradley, Iacopino, & Okunseri, 2008). This matches the population trend in the institute where this study was carried out, as it was reported that a higher number of females (61%) actively sought orthodontic treatment than males (39%). However, the gender inequality was unlikely to be a confounding factor as it has reported that gender did not have a significant influence on the rate of orthodontic tooth movement (Dudic et al., 2013). Additionally, there was no statistically significant association between gender and MOP interval grouping for our study which further excludes gender as a confounding factor.

5.2 Split Mouth Study Design

A split mouth design is useful when conducting a 2 arm parallel randomised clinical trial (RCT) as 2 interventions (either control or intervention) are assessed within the same sample. As both the control and intervention are done on the same sample, this eliminates inter-subject variability related to differences between subjects and provides better comparison between control and intervention (Hujoel & DeRouen, 1992; Ramfjord, Nissle, Shick, & Cooper, 1968).

The limitations of split mouth study designs have been reported by a series of papers by Hujoel's group and Lesaffre's group ((Hujoel & DeRouen, 1992; Hujoel & Loesche,

1990; Hujoel & Moulton, 1988; Lesaffre, Garcia Zattera, Redmond, Huber, & Needleman, 2007; Lesaffre, Philstrom, Needleman, & Worthington, 2009). One of the issues raised was the carry-across effects or "contamination" in which an intervention effect is thought to generally affect the whole body or all sides of the mouth indiscriminately, for example a fluoride mouth wash that will affect all quadrants and prevents segregation of the intervention and control quadrants (Lesaffre et al., 2009). Another disadvantage was thought to be the period effect where interventions are not done simultaneously and this will then affect the results or perception of the intervention, like assessing pain perception of 2 different methods of administrating anaesthesia (Lesaffre et al., 2009). However these concerns which may affect other dental research do not apply to our study as we are measuring the BV/TV and rate of individual canine retraction in different mandibular quadrants of the same patient and using paired T-test statistical analysis which will instead result in decreased variance and higher powered study as compared to a study done with inter-patient subjects (Pandis et al., 2013). The issue of carry-across effect of the MOP is also not like likely to a problem as our results show that the reduction of bone volumetric fraction was limited to the surrounding area around the MOP site, hardly extending pass 14.8mm apically, which would not have crossed over to the opposite mandibular quadrant. In a similar way, the study by Teixeira (Teixeira et al., 2010) had demonstrated that the effect of MOP was limited to the intervention mandibular half and did not cross over to the contralateral side.

5.3 Measurement Method

As noted in the literature review, orthodontic tooth movement occurs due to bone remodelling which is a coupled process of activation, resorption, reversal and formation cycles (Hadjidakis Dimitrios & Androulakis Ioannis, 2007). These coupled remodelling

cycles have an inverse relationship to bone volumetric fraction; the higher the turnover rate of alveolar bone, the lower the BV/TV, and the faster the orthodontic tooth movement (Verna et al., 2000; Verna et al., 1999). An animal study had also demonstrated this relationship where it was reported that MOP cause a reduction in the BV/TV and subsequently faster orthodontic tooth movement (Tsai et al., 2016). In that study, micro-CT was used to measure the bone volume over trabeculae volume or bone volumetric fraction (Tsai et al., 2016). Micro CT can provide images with spatial resolution of 15–20 μm^3 (in contrast our CBCT resolution is 76 µm x 76 µm x 76 µm voxel size), allowing for highly accurate visualisation of trabecular thickness and bone structure (Genant et al., 2000). In order to achieve the very detailed high resolution images, a very high radiation dose is needed, the average dose of micro-CT imaging can be as high as 120 mGy (Cavanaugh et al., 2004). In contrast, the effective dose of an intraoral periapical is about 0.0003mSv (Isaacson & Thom, 2015) while a CBCT with large field of view would be approximately 0.212 mSv (Ludlow et al., 2015). Discounting tissue weighting factors, 1 Gy in dental radiology is equivalent to 1 Sv (Okano & Sur, 2010), which renders the 120 mGy radiation dose of a micro-CT, too high to be used safely for in-vivo studies.

Yip and colleagues suggested that micro CT should be the gold standard in assessing bone trabeculae patterns (Yip et al., 2004). However, the relative merits of high resolution imaging properties should also be considered against its invasiveness; an in-vivo imaging method should prioritise non-invasiveness instead of imaging resolution (Genant et al., 2000). The images taken with CBCT are of lower resolution than micro-CT (76 μm^3 vs 15–20 μm^3) which results in overestimation of small trabeculae and accentuated partial volume effects (Ibrahim et al., 2014), coupled with artefacts from scanning technology (Schulze et al., 2011), effectively consigns CBCT images as poor quality 3D images. However, despite its shortcomings, there was a strong correlation between the trabeculae bone microstructure measurements made by CBCT and micro-CT images with only small

discrepancies between them which suggests that CBCT is an acceptable diagnostic tool to assess bone volumetric fraction and trabeculae bone microstructure (Ibrahim et al., 2014). Therefore, CBCT was used in this study to assess the differences of the bone volumetric fraction of the samples, similar to a study by Chang et al where they used CBCT to evaluate bone trabeculae pattern changes around orthodontically moved teeth (Chang et al., 2012).

5.4 Influence of Micro-osteoperforation on Mandibular Trabeculae Pattern

In our study, the premolar extractions were done once anchorage reinforcement of the molar teeth with temporary anchorage devices were secured. A bumper sleeve was then used to maintain the extraction space while levelling and alignment of the occlusion and subsequent archwire changes were done to enable passive insertion of the working archwire into the bracket slots, only then was canine retraction done and data collected. The period from the start of extractions to initiation of canine retraction is of significant importance as this resting period allows the bone of the extraction socket to heal and settle down. Tooth extraction is a noxious stimuli which causes regional acceleratory phenomenon (RAP) (Verna, 2016), and the resting period "washes out" the RAP effect from tooth extraction and reduces overlapping with MOP induced RAP which can interfere with the study findings.

The usage of split mouth technique allowed the investigation of MOP on the bone volumetric fraction of mandibular alveolar bone without the confounding factors of intra-subject variables. In our study, MOP significantly reduced the mean BV/TV at section V1 (54.18%, SD 14.70) by 9.76% compared to the control site (63.97%, SD 7.64), which concurrently increased the rate of orthodontic tooth movement (Intervention 4.03mm, SD 1.53; control 2.77mm, SD1.33) by 1.25mm during the trial period. This result is in

agreement with 3 animal studies where MOP also significantly reduced the BV/TV while increasing the rate of tooth movement (Cheung et al., 2016; Teixeira et al., 2010; Tsai et al., 2016). Another animal study also reported no difference in the BV/TV subsequent to corticortomy or corticision in rats, which contradicted our study (Librizzi et al., 2017). The explanation by Librizzi et al for the contrast in results were that Librizzi's study used growing rats which had less robust effects from corticotomy while the other studies used mature adult rats. (Librizzi et al., 2017). In those animal studies the BV/TV decreased by 13.46% from 59.48% to 46.02% in the Tsai study, by 5.33% from 81.39% to 76.06% in the Cheung study and by 49% from 82% to 33% in the Teixeira study. The difference in results between those 3 studies and our study could be due to the difference in bone metabolism of animal and humans (Perel et al., 2007). The BV/TV in the animal studies were analysed via the higher resolution micro-CT while our study used CBCT to analyse the BV/TV in human subjects. The lower resolution of CBCT images could result in some minor discrepancies in the calculation of the BV/TV value. Besides that, the accuracy of trabecular bone microstructure measurement with both micro-CT and CBCT are highly dependent on the threshold selection (Parkinson, Badiei, & Fazzalari, 2008), which explains the wide variation in BV/TV values. However as our study was based on the split mouth technique and the threshold value was fixed to be constant for each individual sample (control and intervention side have the same threshold value, each subject had different threshold value), this has allowed the comparison of BV/TV to be more accurate without the influence of other confounding inter-subject variances.

The vertical area of alveolar bone in between the mandibular canine and the mandibular second premolar was divided into 3 vertical sections of 14.8mm each. 14.8mm height was determined to accommodate 3 MOP of 1.6mm diameter each and interdental bone between the MOP site and upper and lower border of each section. Similarly, there was also 3 horizontal sections which coincided with the interdental space

between the roots of the mandibular second incisor, canine, second premolar and first molar. The different sections plotted were used to investigate the BV/TV values horizontally and vertically at both control and intervention sides. Our results show that BV/TV reduced towards the occlusal and increased towards the distal, which is in contrast to the trend reported by 2 other micro-CT studies on human cadavers (Fanuscu & Chang, 2004; Kim et al., 2013). This discrepancy could be due to our sample population undergoing active orthodontic treatment as BV/TV of alveolar bone was reported to decrease during active orthodontic treatment (da Silva Campos et al., 2012; Yu et al., 2016). The larger decrease in BV/TV in the anterior mandible could be because the anterior mandible consist of teeth which were actively being moved as compared to the posterior mandible which contained the molar teeth being indirectly anchored to the miniscrew. Our study also showed that the difference in BV/TV between the horizontal sections were statistically significant for both control and intervention sides. The intervention side had significant lower value at the H2 section but insignificantly lower BV/TV in all other sections as compared to the control side. We hypothesise that the BV/TV reduction effect of MOP has a limited effective range and is not able to affect the whole horizontal section of the mandibular alveolar bone of the respective quadrant. For the BV/TV in the vertical direction, the results of our study which found that MOP induced BV/TV to increase with depth in the corono-apical direction, contradicted with Fanuscu's study, where it was reported that the BV/TV decreased with depth in the corono-apical direction (Fanuscu & Chang, 2004). This pattern of reduction in BV/TV during active orthodontic treatment for the vertical dimension is similar to that in the horizontal dimension, as most orthodontic movements are done near the crestal bone as opposed to near the lower border of the mandible. With the intervention of MOP, the difference in the BV/TV of the vertical sections were statistically significant as opposed to not statistically significant at the control side. The effect of BV/TV reduction by MOP

from V1 into V2 was insufficient to significantly reduce the BV/TV at section V2. This reduction of BV/TV could perhaps be related to faster treatment for alignment of palatally impacted canines as reported by Fischer (Fischer, 2007).

MOP had been reported to significantly reduce the BV/TV of alveolar bone (M. Alikhani et al., 2013; Baloul et al., 2011; Teixeira et al., 2010; Tsai et al., 2016; Verna, 2016). However it was not mentioned as to the extent of bone affected by MOP, hence the attempt to investigate this aspect in our study. The effect of BV/TV reduction by MOP is only significant at the 14.8mm vertical section immediately below the cervical enamel junction of the mandibular canine and the horizontal interdental section between mandibular canine and second premolar of alveolar bone where the MOP was performed. Although some slight decrease in BV/TV was noted at the regions adjacent to the MOP area, they were not statistically significant. This finding was similar to the effect reported in an animal study (Verna et al., 1999) where it was reported that the regional acceleratory phenomenon (RAP) instigated by noxious stimuli from orthodontic treatment caused a statistically significant decrease in the bone fraction values around the second molar area which was not actively under orthodontic force. Another animal study by Teixeira found that the BV/TV reduction effect of MOP was limited to the intervention mandibular half and did not cross over to the contralateral side (Teixeira et al., 2010). Although MOP can significantly reduce the BV/TV of alveolar bone and consequently increase orthodontic tooth movement, its effect is localized to a small area around the MOP and not widely diffused. This means that in order to increase the rate of orthodontic tooth movement of multiple teeth, MOPs at shorter distances have to be done. The use of MOP can also be specifically targeted to increase the rate of orthodontic tooth movement of certain teeth while not affecting the other teeth such as the anchor teeth.

5.5 Influence of Micro-osteoperforation on Rate of Orthodontic Tooth Movement

The rate of orthodontic tooth movement is approximately 1mm per month (Nanda & Kapila, 2010). A wide range of factors such as biomechanical considerations, genetic factors, systemic conditions and age have been reported to influence the rate of orthodontic tooth movement (Dudic et al., 2013; Krishnan & Davidovitch, 2009; Ren et al., 2003). The rate of orthodontic tooth movement can even vary within the same subjects with the same constant orthodontic force applied (Pilon et al., 1996; van Leeuwen, Maltha, & Kuijpers-Jagtman, 1999). This implies that the rate of orthodontic tooth movement is highly subjective and is very much individually determined. MOP was reported to significantly accelerated orthodontic tooth movement by 2.3 times (M. Alikhani et al., 2013), whereas our study only had a 1.45 fold increase of orthodontic tooth movement. The Alikhani's study population consist of 2 separate control and intervention groups which could have some confounding individual variances. Besides that, they used Nickel-titanium (NiTi) closing coil springs reported to produce a more consistent and faster rate of space closure than the elastomeric modules as used in our study (Samuels, Rudge, & Mair, 1998), due to its constant light continuous force (Miura, Mogi, Ohura, & Karibe, 1988), while elastomeric chains are subjected to force decay which reduces the force delivered over time (Halimi, Benyahia, Doukkali, Azeroual, & Zaoui, 2012).

As of October 2018, only 2 systematic reviews (Alfawal, Hajeer, Ajaj, Hamadah, & Brad, 2016; Yi, Xiao, Li, Li, & Zhao, 2017) and one Cochrane review (Fleming et al., 2015) have reviewed the available literature on minimally invasive surgical procedures to accelerate orthodontic treatment. Generally, orthodontic tooth movement was significantly faster with surgical intervention with the pooled results reporting 0.61mm (Fleming et al., 2015) and 0.65mm (Alfawal et al., 2016) more tooth movement in the

first month. The majority of the studies were related to piezocision as the procedure to accelerate orthodontic tooth movement. Only one study (M. Alikhani et al., 2013) in those 3 systematic reviews involved micro-osteoperforations. Our study reported an increased rate of orthodontic tooth movement of 1.25mm over 3 months which averages out to 0.4mm for 1 month. As orthodontic tooth movement rate is highly variable and is affected by a multitude of external and internal factors, this can be related to the slight discrepancy in the rates of tooth movement to our study.

5.6 Influence of Micro-osteoperforation Intervals on Mandibular Trabeculae Pattern Related to the Rate of Orthodontic Tooth Movement

Our study is the first randomised clinical trial to investigate the effectiveness of MOP with regards to its effective range and the effective MOP interval. Both are important considerations in the clinical application of MOP to accelerate orthodontic tooth movement. When our data was analysed based on the MOP intervals of 4, 8 and 12 weeks, the BV/TV trend at 4 weekly interval was similar to our result as a whole. The BV/TV at V1 and H2 showed statistically significant reduction by almost 15% while the rest of the sites showed a statistically insignificant reduction. The horizontal and vertical BV/TV trends were also similar where it decreased as it extended coronally and mesially. However this was not the case for both the 8 and 12 weekly MOP intervals where the mean BV/TV difference between all control and intervention sites were not statistically significant except for the reduction in BV/TV of intervention side H2 at the 12 weekly MOP interval. RAP occurs a few hours after noxious stimulation with the initiation of the inflammatory process, then it achieves peak effect at 1-2 months and lasts for about 4 months in bone (Schilling et al., 1998; Wilcko et al., 2001). It was recommended that orthodontic movements should be commenced not later than 2 weeks after the accelerated

orthodontic intervention as to fully utilise the RAP effect which will last about 4-6 months (Amit et al., 2012; K. G. Murphy, Wilcko, Wilcko, & Ferguson, 2009). The peak effect of RAP was seen in the subjects who had MOP interventions at 4 weekly interval which coincided with significant reduction in BV/TV. The reduction in BV/TV was less pronounced with the 8 and 12 weekly intervals probably due to RAP effect not being at peak values.

Despite only the 4 weekly MOP interval showing a statistically significant difference in the BV/TV between control and intervention side of V1 (where the active orthodontic canine movement was occurring), all 3 MOP intervals showed statistically significant difference for the canine retraction rate with the intervention side having increased tooth movement by almost 0.3mm per month. The 4 weekly MOP intervention posted the highest mean difference with the intervention side quicker by 1.49mm over the trial duration of 4 weeks. As the RAP induced by MOP is thought to decrease BV/TV and increase alveolar bone turnover, which in turn accelerates orthodontic tooth movement (Goldie & King, 1984; Schilling et al., 1998), this could imply that the peak effect of RAP is needed to cause a significant reduction of BV/TV, however significantly increased rates of orthodontic tooth movement can be achieved as long as the RAP effect is present, even at non-peak levels. Interestingly, our study also showed a moderate negative Pearson correlation between the BV/TV of V1 and the rate of canine movement, which indicates that the lower the BV/TV, the faster the orthodontic tooth movement. This is in agreement with an animal study by Goldie and King where they reported increased orthodontic tooth movement in rats with osteoporosis (Goldie & King, 1984).

5.7 Limitations and Recommendations

It is also important to consider that bone remodelling for orthodontic tooth movement is a coupled cyclic process that involves a multitude of chemical and biological processes that can be influenced by a variety of mediators (Hadjidakis Dimitrios & Androulakis Ioannis, 2007; H. Huang et al., 2014; Krishnan & Davidovitch, 2006, 2009; Melsen, 1999, 2001; Verna, 2016; Verna et al., 1999; Wilcko et al., 2001). Hence, it is safe to assume that the mandibular trabeculae pattern exists in waves of fluctuating BV/TV values. For ethical reasons, the CBCT was taken only at 1 time point, and this unfortunately only provides a cross-sectional view of the BV/TV at that time point and cannot represent a complete picture of a continuous process. This limitation is reflected in our study results where the BV/TV showed significant difference for the 4 weekly MOP interval but not significant for the 8 and 12 weekly MOP intervals despite all 3 interval periods showing significantly faster canine retraction rates.

The resolution for our CBCT images were 76 μm^3 compared to the resolution of 15–20 μm^3 for micro-CT images which is a huge difference in image resolution quality. Despite studies that show strong correlations between the trabeculae bone microstructure measurements made by CBCT and micro-CT image (Ibrahim et al., 2014), it cannot be denied that higher resolution images provide better quality images for analysis purposes. The drawbacks of a smaller CBCT voxel size is the reduced contrast-to-noise ratio (CNR) (Bechara et al., 2012). CNR is a quantitative image quality parameter which influences the quality of a radiographic or CBCT image (Taylor, 2016). Radiographic images with higher CNR will provide better visualisation of the fine trabeculae microstructures and more accurate BV/TV measurements. This shortcoming is reflected in our study where our results show insignificant differences in the BV/TV of the 8 and 12 week MOP interval groups despite significantly faster canine retraction rates for both groups. Our results even showed a moderately significant correlation between BV/TV and rate of

canine retraction. In addition, the dropouts which reduced the sample size of the 8 week MOP group to below the targeted sample size may have also been underpowered to detect the difference. Therefore it is our recommendation that in future studies, the sample size be increased or imaging technologies with better resolution but reduced radiation be used in order to compensate for the lack of sensitivity of CBCT images in BV/TV detection.

Our study showed that 4 weekly MOP intervals induced a significant localised reduction in BV/TV around the MOP site as compared to the other intervention intervals, and had significant acceleration of tooth movement. 8 and 12 weekly MOP intervals had significant increase in orthodontic tooth movement but insignificant reduction in BV/TV. Before further evidence can be elicited from bigger sampled studies, we therefore recommend that 4 weekly MOP intervention be performed in certain clinical situations which may benefit from a reduction in BV/TV such as traction of unerupted teeth or protracting posterior teeth.

CHAPTER 6: CONCLUSION

This study investigated the effects of micro-osteoperforation (MOP) on the horizontal and vertical distribution of mandibular trabeculae pattern in relation to different MOP intervals and on canine retraction. Based on the findings and limitations of the study, we conclude that:-

i. There is significant reduction of the mandibular trabecular alveolar bone volume fraction at the MOP as compared to the control sides, the reduction limited to the immediate interdental area of the intervention site.
ii. MOP reduction of mandibular trabecular alveolar bone volume fraction at the immediate interdental area of the intervention site is significant only for the 4 weekly MOP interval, even though the rate of canine movement was significantly increased for all intervention intervals.
iii. The mandibular trabecular alveolar bone volume fraction significantly decreased with MOP intervention as the rate of canine tooth movement significantly increased, the inverse linear association is of moderate strength.
iv. The effective range of BV/TV reduction has not been proven to extend beyond the interdental region where the MOP was performed.

REFERENCES

Ackerman, J. L., & Proffit, W. R. (1997). Soft tissue limitations in orthodontics: treatment planning guidelines. *Angle Orthod, 67*(5), 327-336. doi:10.1043/0003-3219(1997)067<0327:stliot>2.3.co;2

Alfawal, A. M., Hajeer, M. Y., Ajaj, M. A., Hamadah, O., & Brad, B. (2016). Effectiveness of minimally invasive surgical procedures in the acceleration of tooth movement: a systematic review and meta-analysis. *Prog Orthod, 17*(1), 33. doi:10.1186/s40510-016-0146-9

Alikhani, M., Alansari, S., Sangsuwon, C., Alikhani, M., Chou, M. Y., Alyami, B., . . . Teixeira, C. C. (2015). Micro-osteoperforations: Minimally invasive accelerated tooth movement. *Seminars in Orthodontics, 21*(3), 162-169. doi:https://doi.org/10.1053/j.sodo.2015.06.002

Alikhani, M., Khoo, E., Alyami, B., Raptis, M., Salgueiro, J. M., Oliveira, S. M., . . . Teixeira, C. C. (2012). Osteogenic effect of high-frequency acceleration on alveolar bone. *Journal of Dental Research, 91*(4), 413-419. doi:10.1177/0022034512438590

Alikhani, M., Raptis, M., Zoldan, B., Sangsuwon, C., Lee, Y. B., Alyami, B., . . . Teixeira, C. (2013). Effect of micro-osteoperforations on the rate of tooth movement. *Am J Orthod Dentofacial Orthop, 144*(5), 639-648. doi:10.1016/j.ajodo.2013.06.017

Amit, G., Jps, K., Pankaj, B., Suchinder, S., & Parul, B. (2012). Periodontally accelerated osteogenic orthodontics (PAOO) - a review. *Journal of Clinical and Experimental Dentistry, 4*(5), e292-e296. doi:10.4317/jced.50822

An, J., Li, Y., Liu, Z., Wang, R., & Zhang, B. (2017). A micro-CT study of microstructure change of alveolar bone during orthodontic tooth movement under different force magnitudes in rats. *Exp Ther Med, 13*(5), 1793-1798. doi:10.3892/etm.2017.4186

Angamuthu, M., & Ramalingam, A. (2011). Balanced key tree management for multi-privileged groups using (N, T) policy. *Security and Communication Networks*.

Angle, E. H. (1907). *Treatment of Malocclusion of the Teeth: Angle's System. 7th Ed., Greatly Enl. and Entirely Rewritten, with Six Hundred and Forty-one Illustrations*: S.S. White Dental Manufacturing Company.

Baloul, S. S. (2016). Osteoclastogenesis and Osteogenesis during Tooth Movement. *Front Oral Biol, 18*, 75-79. doi:10.1159/000351901

Baloul, S. S., Gerstenfeld, L. C., Morgan, E. F., Carvalho, R. S., Van Dyke, T. E., & Kantarci, A. (2011). Mechanism of action and morphologic changes in the alveolar bone in response to selective alveolar decortication-facilitated tooth movement. *Am J Orthod Dentofacial Orthop, 139*(4 Suppl), S83-101. doi:10.1016/j.ajodo.2010.09.026

Bechara, B., McMahan, C. A., Moore, W. S., Noujeim, M., Geha, H., & Teixeira, F. B. (2012). Contrast-to-noise ratio difference in small field of view cone beam computed tomography machines. *J Oral Sci, 54*(3), 227-232.

Bell, M. L., Kenward, M. G., Fairclough, D. L., & Horton, N. J. (2013). Differential dropout and bias in randomised controlled trials: when it matters and when it may not. *BMJ : British Medical Journal, 346*.

Bruker. (2013). CT Analyser User Manual Version 1.13. Retrieved from http://bruker-microct.com website:

Burstone, C. J. (1962). The biomechanics of tooth movement. *Vistas in orthodontics*, 197-213.

Buschang, P. H., Campbell, P. M., & Ruso, S. (2012). Accelerating Tooth Movement With Corticotomies: Is It Possible and Desirable? *Seminars in Orthodontics, 18*(4), 286-294. doi:https://doi.org/10.1053/j.sodo.2012.06.007

Campos, M. J., de Souza, T. S., Mota Junior, S. L., Fraga, M. R., & Vitral, R. W. (2014). Bone mineral density in cone beam computed tomography: Only a few shades of gray. *World J Radiol, 6*(8), 607-612. doi:10.4329/wjr.v6.i8.607

Cassetta, M., Stefanelli, L. V., Pacifici, A., Pacifici, L., & Barbato, E. (2014). How accurate is CBCT in measuring bone density? A comparative CBCT-CT in vitro study. *Clin Implant Dent Relat Res, 16*(4), 471-478. doi:10.1111/cid.12027

Cavanaugh, D., Johnson, E., Price, R. E., Kurie, J., Travis, E. L., & Cody, D. D. (2004). In vivo respiratory-gated micro-CT imaging in small-animal oncology models. *Mol Imaging, 3*(1), 55-62. doi:10.1162/153535004773861723

Chang, H.-W., Huang, H.-L., Yu, J.-H., Hsu, J.-T., Li, Y.-F., & Wu, Y.-F. (2012). Effects of orthodontic tooth movement on alveolar bone density. *Clinical Oral Investigations, 16*(3), 679-688. doi:10.1007/s00784-011-0552-9

Cheung, T., Park, J., Lee, D., Kim, C., Olson, J., Javadi, S., . . . Hong, C. (2016). Ability of Mini-Implant Facilitated Micro-osteoperforations to Accelerate Tooth Movement in Rats. *Am J Orthod Dentofacial Orthop, 150*(6), 958-967. doi:10.1016/j.ajodo.2016.04.030

Cohen, G., Campbell, P. M., Rossouw, P. E., & Buschang, P. H. (2010). Effects of increased surgical trauma on rates of tooth movement and apical root resorption in foxhound dogs. *Orthod Craniofac Res, 13*(3), 179-190. doi:10.1111/j.1601-6343.2010.01494.x

da Silva Campos, M. J., de Albuquerque, E. G., Pinto, B. C. H., Hungaro, H. M., Gravina, M. A., Fraga, M. R., & Vitral, R. W. F. (2012). The role of orthodontic tooth movement in bone and root mineral density: A study of patients submitted and not submitted to orthodontic treatment. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research, 18*(12), CR752-CR757. doi:10.12659/MSM.883604

Dudic, A., Giannopoulou, C., & Kiliaridis, S. (2013). Factors related to the rate of orthodontically induced tooth movement. *Am J Orthod Dentofacial Orthop, 143*(5), 616-621. doi:10.1016/j.ajodo.2012.12.009

Eckstein, F., Matsuura, M., Kuhn, V., Priemel, M., Muller, R., Link, T. M., & Lochmuller, E. M. (2007). Sex differences of human trabecular bone microstructure in aging are site-dependent. *J Bone Miner Res, 22*(6), 817-824. doi:10.1359/jbmr.070301

El-Angbawi, A., McIntyre, G. T., Fleming, P. S., & Bearn, D. R. (2015). Non-surgical adjunctive interventions for accelerating tooth movement in patients undergoing fixed orthodontic treatment. *Cochrane Database Syst Rev*(11), Cd010887. doi:10.1002/14651858.CD010887.pub2

Fanuscu, M. I., & Chang, T. L. (2004). Three-dimensional morphometric analysis of human cadaver bone: microstructural data from maxilla and mandible. *Clin Oral Implants Res, 15*(2), 213-218.

Faot, F., Chatterjee, M., de Camargos, G. V., Duyck, J., & Vandamme, K. (2015). Micro-CT analysis of the rodent jaw bone micro-architecture: A systematic review. *Bone Rep, 2*, 14-24. doi:10.1016/j.bonr.2014.10.005

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. J. B. R. M. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *39*(2), 175-191. doi:10.3758/bf03193146

Fischer, T. J. (2007). Orthodontic treatment acceleration with corticotomy-assisted exposure of palatally impacted canines. *Angle Orthod, 77*(3), 417-420. doi:10.2319/0003-3219(2007)077[0417:otawce]2.0.co;2

Fisher, R. J. O. B., Edinburgh. (1954). Statistical Methods for Research Workers, Section 21.1.

Fleming, P. S., Fedorowicz, Z., Johal, A., El-Angbawi, A., & Pandis, N. (2015). Surgical adjunctive procedures for accelerating orthodontic treatment. *Cochrane Database Syst Rev*(6), Cd010572. doi:10.1002/14651858.CD010572.pub2

Friedman, A. W. (2006). Important determinants of bone strength: beyond bone mineral density. *J Clin Rheumatol, 12*(2), 70-77. doi:10.1097/01.rhu.0000208612.33819.8c

Frost, H. M. (1983). The regional acceleratory phenomenon: a review. *Henry Ford Hosp Med J, 31*(1), 3-9.

Frost, H. M. (1994). Wolff's Law and bone's structural adaptations to mechanical usage: an overview for clinicians. *The Angle Orthodontist, 64*(3), 175-188. doi:10.1043/0003-3219(1994)064<0175:wlabsa>2.0.co;2

Fyhrie, D. P. (2005). Summary--Measuring "bone quality". *J Musculoskelet Neuronal Interact, 5*(4), 318-320.

Genant, H. K., Engelke, K., Fuerst, T., Gluer, C. C., Grampp, S., Harris, S. T., . . . Takada, M. (1996). Noninvasive assessment of bone mineral and structure: state of the art. *J Bone Miner Res, 11*(6), 707-730. doi:10.1002/jbmr.5650110602

Genant, H. K., Engelke, K., & Prevrhal, S. (2008). Advanced CT bone imaging in osteoporosis. *Rheumatology (Oxford), 47 Suppl 4*, iv9-16. doi:10.1093/rheumatology/ken180

Genant, H. K., Gordon, C., Jiang, Y., Link, T. M., Hans, D., Majumdar, S., & Lang, T. F. (2000). Advanced imaging of the macrostructure and microstructure of bone. *Horm Res, 54 Suppl 1*, 24-30. doi:10.1159/000063444

Ghasemi, A., & Zahediasl, S. (2012). Normality Tests for Statistical Analysis: A Guide for Non-Statisticians. *International Journal of Endocrinology and Metabolism, 10*(2), 486-489. doi:10.5812/ijem.3505

Goldie, R. S., & King, G. J. (1984). Root resorption and tooth movement in orthodontically treated, calcium-deficient, and lactating rats. *Am J Orthod, 85*(5), 424-430.

Gomes de Oliveira, R. C., Leles, C. R., Lindh, C., & Ribeiro-Rotta, R. F. (2012). Bone tissue microarchitectural characteristics at dental implant sites. Part 1: Identification of clinical-related parameters. *23*(8), 981-986. doi:10.1111/j.1600-0501.2011.02243.x

Gonzalez-Garcia, R., & Monje, F. (2013). The reliability of cone-beam computed tomography to assess bone density at dental implant recipient sites: a histomorphometric analysis by micro-CT. *Clin Oral Implants Res, 24*(8), 871-879. doi:10.1111/j.1600-0501.2011.02390.x

Hadjidakis Dimitrios, J., & Androulakis Ioannis, I. (2007). Bone Remodeling. *Annals of the New York Academy of Sciences, 1092*(1), 385-396. doi:10.1196/annals.1365.035

Halimi, A., Benyahia, H., Doukkali, A., Azeroual, M. F., & Zaoui, F. (2012). A systematic review of force decay in orthodontic elastomeric power chains. *Int Orthod, 10*(3), 223-240. doi:10.1016/j.ortho.2012.06.013

Hatcher, D. C. (2010). Operational principles for cone-beam computed tomography. *J Am Dent Assoc, 141 Suppl 3*, 3s-6s.

Hsu, J.-T., Chang, H.-W., Huang, H.-L., Yu, J.-H., Li, Y.-F., & Tu, M.-G. J. C. O. I. (2011). Bone density changes around teeth during orthodontic treatment. *15*(4), 511-519. doi:10.1007/s00784-010-0410-1

Huang, H., Williams, R. C., & Kyrkanides, S. (2014). Accelerated orthodontic tooth movement: molecular mechanisms. *Am J Orthod Dentofacial Orthop, 146*(5), 620-632. doi:10.1016/j.ajodo.2014.07.007

Huang, Y., Dessel, J. V., Depypere, M., EzEldeen, M., Iliescu, A. A., Santos, E. D., . . . Jacobs, R. (2014). Validating cone-beam computed tomography for peri-implant bone morphometric analysis. *Bone research, 2*, 14010-14010. doi:10.1038/boneres.2014.10

Hujoel, P. P., & DeRouen, T. A. (1992). Validity issues in split-mouth trials. *J Clin Periodontol, 19*(9 Pt 1), 625-627.

Hujoel, P. P., & Loesche, W. J. (1990). Efficiency of split-mouth designs. *J Clin Periodontol, 17*(10), 722-728.

Hujoel, P. P., & Moulton, L. H. (1988). Evaluation of test statistics in split-mouth clinical trials. *J Periodontal Res, 23*(6), 378-380.

Ibrahim, N., Parsa, A., Hassan, B., van der Stelt, P., Aartman, I. H., & Wismeijer, D. (2014). Accuracy of trabecular bone microstructural measurement at planned dental implant sites using cone-beam CT datasets. *Clin Oral Implants Res, 25*(8), 941-945. doi:10.1111/clr.12163

Isaacson, K., & Thom, A. R. (2015). Orthodontic radiography guidelines. *American Journal of Orthodontics and Dentofacial Orthopedics, 147*(3), 295-296. doi:10.1016/j.ajodo.2014.12.005

Kazama, J. J., Koda, R., Yamamoto, S., Narita, I., Gejyo, F., & Tokumoto, A. (2010). Cancellous Bone Volume Is an Indicator for Trabecular Bone Connectivity in Dialysis Patients. *Clinical Journal of the American Society of Nephrology : CJASN, 5*(2), 292-298. doi:10.2215/CJN.04150609

Khosla, S., & Riggs, B. L. (2005). Pathophysiology of age-related bone loss and osteoporosis. *Endocrinol Metab Clin North Am, 34*(4), 1015-1030, xi. doi:10.1016/j.ecl.2005.07.009

Kim, J. E., Shin, J. M., Oh, S. O., Yi, W. J., Heo, M. S., Lee, S. S., . . . Huh, K. H. (2013). The three-dimensional microstructure of trabecular bone: Analysis of site-specific variation in the human jaw bone. *Imaging Sci Dent, 43*(4), 227-233. doi:10.5624/isd.2013.43.4.227

King, G. J., Keeling, S. D., & Wronski, T. J. (1991). Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. *Bone, 12*(6), 401-409.

Koletsi, D., Pandis, N., & Fleming, P. S. (2014). Sample size in orthodontic randomized controlled trials: are numbers justified? *Eur J Orthod, 36*(1), 67-73. doi:10.1093/ejo/cjt005

Koo, T. K., & Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of chiropractic medicine, 15*(2), 155-163. doi:10.1016/j.jcm.2016.02.012

Krishnan, V., & Davidovitch, Z. (2006). Cellular, molecular, and tissue-level reactions to orthodontic force. *Am J Orthod Dentofacial Orthop, 129*(4), 469.e461-432. doi:10.1016/j.ajodo.2005.10.007

Krishnan, V., & Davidovitch, Z. (2009). On a path to unfolding the biological mechanisms of orthodontic tooth movement. *J Dent Res, 88*(7), 597-608. doi:10.1177/0022034509338914

Legrand, E., Chappard, D., Pascaretti, C., Duquenne, M., Krebs, S., Rohmer, V., . . . Audran, M. (2000). Trabecular bone microarchitecture, bone mineral density, and vertebral fractures in male osteoporosis. *J Bone Miner Res, 15*(1), 13-19. doi:10.1359/jbmr.2000.15.1.13

Lesaffre, E., Garcia Zattera, M. J., Redmond, C., Huber, H., & Needleman, I. (2007). Reported methodological quality of split-mouth studies. *J Clin Periodontol, 34*(9), 756-761. doi:10.1111/j.1600-051X.2007.01118.x

Lesaffre, E., Philstrom, B., Needleman, I., & Worthington, H. (2009). The design and analysis of split-mouth studies: what statisticians and clinicians should know. *Stat Med, 28*(28), 3470-3482. doi:10.1002/sim.3634

Librizzi, Z., Kalajzic, Z., Camacho, D., Yadav, S., Nanda, R., & Uribe, F. (2017). Comparison of the effects of three surgical techniques on the rate of orthodontic tooth movement in a rat model. *The Angle Orthodontist, 87*(5), 717-724. doi:10.2319/123016-940.1

Ludlow, J. B., Davies-Ludlow, L. E., Brooks, S. L., & Howerton, W. B. (2006). Dosimetry of 3 CBCT devices for oral and maxillofacial radiology: CB Mercuray, NewTom 3G and i-CAT. *Dentomaxillofac Radiol, 35*(4), 219-226. doi:10.1259/dmfr/14340323

Ludlow, J. B., Timothy, R., Walker, C., Hunter, R., Benavides, E., Samuelson, D. B., & Scheske, M. J. (2015). Effective dose of dental CBCT—a meta analysis of published data and additional data for nine CBCT units. *Dentomaxillofacial Radiology, 44*(1), 20140197. doi:10.1259/dmfr.20140197

Mavreas, D., & Athanasiou, A. E. (2008). Factors affecting the duration of orthodontic treatment: a systematic review. *Eur J Orthod, 30*(4), 386-395. doi:10.1093/ejo/cjn018

Melsen, B. (1999). Biological reaction of alveolar bone to orthodontic tooth movement. *Angle Orthod, 69*(2), 151-158. doi:10.1043/0003-3219(1999)069<0151:broabt>2.3.co;2

Melsen, B. (2001). Tissue reaction to orthodontic tooth movement--a new paradigm. *Eur J Orthod, 23*(6), 671-681.

Midgett, R. J., Shaye, R., & Fruge, J. F., Jr. (1981). The effect of altered bone metabolism on orthodontic tooth movement. *Am J Orthod, 80*(3), 256-262.

Minkin, C., & Marinho, V. C. (1999). Role of the osteoclast at the bone-implant interface. *Adv Dent Res, 13*, 49-56. doi:10.1177/08959374990130011401

Miura, F., Mogi, M., Ohura, Y., & Karibe, M. (1988). The super-elastic Japanese NiTi alloy wire for use in orthodontics. Part III. Studies on the Japanese NiTi alloy coil springs. *Am J Orthod Dentofacial Orthop, 94*(2), 89-96.

Molteni, R. (2013). Prospects and challenges of rendering tissue density in Hounsfield units for cone beam computed tomography. *Oral Surg Oral Med Oral Pathol Oral Radiol, 116*(1), 105-119. doi:10.1016/j.oooo.2013.04.013

Muller, R., Koller, B., Hildebrand, T., Laib, A., Gianolini, S., & Ruegsegger, P. (1996). Resolution dependency of microstructural properties of cancellous bone based on three-dimensional mu-tomography. *Technol Health Care, 4*(1), 113-119.

Murphy, C. A., Chandhoke, T., Kalajzic, Z., Flynn, R., Utreja, A., Wadhwa, S., . . . Uribe, F. (2014). Effect of corticision and different force magnitudes on orthodontic tooth movement in a rat model. *Am J Orthod Dentofacial Orthop, 146*(1), 55-66. doi:10.1016/j.ajodo.2014.03.024

Murphy, K. G., Wilcko, M. T., Wilcko, W. M., & Ferguson, D. J. (2009). Periodontal accelerated osteogenic orthodontics: a description of the surgical technique. *J Oral Maxillofac Surg, 67*(10), 2160-2166. doi:10.1016/j.joms.2009.04.124

Nanda, R., & Kapila, S. D. (2010). *Current Therapy in Orthodontics*: Mosby Elsevier.

Nimeri, G., Kau, C. H., Abou-Kheir, N. S., & Corona, R. (2013). Acceleration of tooth movement during orthodontic treatment--a frontier in orthodontics. *Prog Orthod, 14*, 42. doi:10.1186/2196-1042-14-42

Okano, T., & Sur, J. (2010). Radiation dose and protection in dentistry. *Japanese Dental Science Review, 46*(2), 112-121. doi:https://doi.org/10.1016/j.jdsr.2009.11.004

Pandis, N. (2012). Sample calculation for split-mouth designs. *American Journal of Orthodontics and Dentofacial Orthopedics, 141*(6), 818-819. doi:10.1016/j.ajodo.2012.03.015

Pandis, N. (2015). Comparison of 2 means for matched observations (paired t test) and t test assumptions. *American Journal of Orthodontics and Dentofacial Orthopedics, 148*(3), 515-516. doi:10.1016/j.ajodo.2015.06.011

Pandis, N., Walsh, T., Polychronopoulou, A., Katsaros, C., & Eliades, T. (2013). Split-mouth designs in orthodontics: an overview with applications to orthodontic clinical trials. *Eur J Orthod, 35*(6), 783-789. doi:10.1093/ejo/cjs108

Parkinson, I. H., Badiei, A., & Fazzalari, N. L. (2008). Variation in segmentation of bone from micro-CT imaging: implications for quantitative morphometric analysis. *Australas Phys Eng Sci Med, 31*(2), 160-164.

Parsa, A., Ibrahim, N., Hassan, B., van der Stelt, P., & Wismeijer, D. (2015). Bone quality evaluation at dental implant site using multislice CT, micro-CT, and cone beam CT. *Clin Oral Implants Res, 26*(1), e1-7. doi:10.1111/clr.12315

Perel, P., Roberts, I., Sena, E., Wheble, P., Briscoe, C., Sandercock, P., . . . Khan, K. S. (2007). Comparison of treatment effects between animal experiments and clinical trials: systematic review. *Bmj, 334*(7586), 197. doi:10.1136/bmj.39048.407928.BE

Pilon, J. J., Kuijpers-Jagtman, A. M., & Maltha, J. C. (1996). Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. *Am J Orthod Dentofacial Orthop, 110*(1), 16-23.

Proffit, W. R. (1978). Equilibrium theory revisited: factors influencing position of the teeth. *Angle Orthod, 48*(3), 175-186. doi:10.1043/0003-3219(1978)048<0175:etrfip>2.0.co;2

Raisz, L. G. (1999). Physiology and pathophysiology of bone remodeling. *Clin Chem, 45*(8 Pt 2), 1353-1358.

Raisz, L. G., & Seeman, E. (2001). Causes of age-related bone loss and bone fragility: an alternative view. *J Bone Miner Res, 16*(11), 1948-1952. doi:10.1359/jbmr.2001.16.11.1948

Ralston, S. H. (2017). Bone structure and metabolism. *Medicine, 45*(9), 560-564. doi:10.1016/j.mpmed.2017.06.008

Ramfjord, S. P., Nissle, R. R., Shick, R. A., & Cooper, H., Jr. (1968). Subgingival curettage versus surgical elimination of periodontal pockets. *J Periodontol, 39*(3), 167-175.

Reitan, K. (1967). Clinical and histologic observations on tooth movement during and after orthodontic treatment. *Am J Orthod, 53*(10), 721-745.

Ren, Y., Maltha, J. C., & Kuijpers-Jagtman, A. M. (2003). Optimum force magnitude for orthodontic tooth movement: a systematic literature review. *Angle Orthod, 73*(1), 86-92. doi:10.1043/0003-3219(2003)073<0086:ofmfot>2.0.co;2

Roberts, W. E., Huja, S., & Roberts, J. A. (2004). Bone modeling: biomechanics, molecular mechanisms, and clinical perspectives. *Seminars in Orthodontics, 10*(2), 123-161. doi:10.1053/j.sodo.2004.01.003

Rosen, C. J., Donahue, L. R., & Hunter, S. J. (1994). Insulin-like growth factors and bone: the osteoporosis connection. *Proc Soc Exp Biol Med, 206*(2), 83-102.

Rubin, D. B. (1976). Inference and missing data. *Biometrika, 63*(3), 581-592. doi:10.1093/biomet/63.3.581

Rygh, P. (1974). Elimination of hyalinized periodontal tissues associated with orthodontic tooth movement. *Scand J Dent Res, 82*(1), 57-73.

Sakka, S., & Coulthard, P. (2009). Bone quality: a reality for the process of osseointegration. *Implant Dent, 18*(6), 480-485. doi:10.1097/ID.0b013e3181bb840d

Samuels, R. H., Rudge, S. J., & Mair, L. H. (1998). A clinical study of space closure with nickel-titanium closed coil springs and an elastic module. *Am J Orthod Dentofacial Orthop, 114*(1), 73-79.

Sangsuwon, C., Alansari, S., Lee, Y. b., Nervina, J., & Alikhani, M. (2017). Step-by-Step Guide for Performing Micro-osteoperforations. In M. Alikhani (Ed.), *Clinical Guide to Accelerated Orthodontics: With a Focus on Micro-Osteoperforations* (pp. 99-116). Cham: Springer International Publishing.

Sangsuwon, C., Alansari, S., Nervina, J., Teixeira, C. C., & Alikhani, M. J. C. D. R. (2017). Micro-osteoperforations in accelerated orthodontics. *2*(1), 4. doi:10.1007/s41894-017-0013-1

Scarfe, W. C., & Farman, A. G. (2008). What is cone-beam CT and how does it work? *Dent Clin North Am, 52*(4), 707-730, v. doi:10.1016/j.cden.2008.05.005

Scarfe, W. C., Farman, A. G., & Sukovic, P. (2006). Clinical applications of cone-beam computed tomography in dental practice. *J Can Dent Assoc, 72*(1), 75-80.

Schilling, T., Muller, M., Minne, H. W., & Ziegler, R. (1998). Influence of inflammation-mediated osteopenia on the regional acceleratory phenomenon and the systemic acceleratory phenomenon during healing of a bone defect in the rat. *Calcif Tissue Int, 63*(2), 160-166.

Schulze, R., Heil, U., Gross, D., Bruellmann, D. D., Dranischnikow, E., Schwanecke, U., & Schoemer, E. (2011). Artefacts in CBCT: a review. *Dentomaxillofac Radiol, 40*(5), 265-273. doi:10.1259/dmfr/30642039

Schwarz, A. M. (1932). Tissue changes incidental to orthodontic tooth movement. *International Journal of Orthodontia, Oral Surgery and Radiography, 18*(4), 331-352. doi:https://doi.org/10.1016/S0099-6963(32)80074-8

Sebaoun, J.-D., Kantarci, A., Turner, J. W., Carvalho, R. S., Van Dyke, T. E., & Ferguson, D. J. (2008). Modeling of Trabecular Bone and Lamina Dura Following Selective Alveolar Decortication in Rats. *J Periodontol, 79*(9), 1679-1688. doi:10.1902/jop.2008.080024

Shih, M. S., & Norrdin, R. W. (1985). Regional acceleration of remodeling during healing of bone defects in beagles of various ages. *Bone, 6*(5), 377-379.

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability. *Psychol Bull, 86*(2), 420-428.

Sivarajan, S., Doss, J. G., Papageorgiou, S. N., Cobourne, M. T., & Wey, M. C. (2018). Mini-implant supported canine retraction with micro-osteoperforation: A split-mouth exploratory randomized clinical trial. *Angle Orthod*. doi:10.2319/011518-47.1

Sugimori, T., Yamaguchi, M., Shimizu, M., Kikuta, J., Hikida, T., Hikida, M., . . . Kasai, K. (2018). Micro-osteoperforations accelerate orthodontic tooth movement by stimulating periodontal ligament cell cycles. *Am J Orthod Dentofacial Orthop, 154*(6), 788-796. doi:10.1016/j.ajodo.2018.01.023

Taylor, C. (2016). Evaluation of the effects of positioning and configuration on contrast-to-noise ratio in the quality control of a 3D Accuitomo 170 dental CBCT system. *Dentomaxillofac Radiol, 45*(5), 20150430-20150430. doi:10.1259/dmfr.20150430

Teixeira, C. C., Khoo, E., Tran, J., Chartres, I., Liu, Y., Thant, L. M., . . . Alikhani, M. (2010). Cytokine Expression and Accelerated Tooth Movement. *Journal of Dental Research, 89*(10), 1135-1141. doi:10.1177/0022034510373764

Travess, H., Roberts-Harry, D., & Sandy, J. (2004). Orthodontics. Part 6: Risks in orthodontic treatment. *Br Dent J, 196*(2), 71-77. doi:10.1038/sj.bdj.4810891

Tsai, C.-Y., Yang, T.-K., Hsieh, H.-Y., & Yang, L.-Y. (2016). Comparison of the effects of micro-osteoperforation and corticision on the rate of orthodontic tooth movement in rats. *The Angle Orthodontist, 86*(4), 558-564. doi:10.2319/052015-343.1

Tsichlaki, A., Chin, S. Y., Pandis, N., & Fleming, P. S. (2016). How long does treatment with fixed orthodontic appliances last? A systematic review. *Am J Orthod Dentofacial Orthop, 149*(3), 308-318. doi:10.1016/j.ajodo.2015.09.020

Van Dessel, J., Huang, Y., Depypere, M., Rubira-Bullen, I., Maes, F., & Jacobs, R. (2013). A comparative evaluation of cone beam CT and micro-CT on trabecular bone structures in the human mandible. *Dentomaxillofac Radiol, 42*(8), 20130145. doi:10.1259/dmfr.20130145

Van Dessel, J., Nicolielo, L. F., Huang, Y., Coudyzer, W., Salmon, B., Lambrichts, I., & Jacobs, R. (2017). Accuracy and reliability of different cone beam computed tomography (CBCT) devices for structural analysis of alveolar bone in comparison with multislice CT and micro-CT. *Eur J Oral Implantol, 10*(1), 95-105.

van Leeuwen, E. J., Maltha, J. C., & Kuijpers-Jagtman, A. M. (1999). Tooth movement with light continuous and discontinuous forces in beagle dogs. *Eur J Oral Sci, 107*(6), 468-474.

Verna, C. (2016). Regional Acceleratory Phenomenon. *Front Oral Biol, 18*, 28-35. doi:10.1159/000351897

Verna, C., Dalstra, M., & Melsen, B. (2000). The rate and the type of orthodontic tooth movement is influenced by bone turnover in a rat model. *Eur J Orthod, 22*(4), 343-352.

Verna, C., Zaffe, D., & Siciliani, G. (1999). Histomorphometric study of bone reactions during orthodontic tooth movement in rats. *Bone, 24*(4), 371-379.

von Bohl, M., & Kuijpers-Jagtman, A. M. (2009). Hyalinization during orthodontic tooth movement: a systematic review on tissue reactions. *Eur J Orthod, 31*(1), 30-36. doi:10.1093/ejo/cjn080

Whaites, E., & Drage, N. (2013). Essentials of Dental Radiography and Radiology.

Whitesides, J., Pajewski, N. M., Bradley, T. G., Iacopino, A. M., & Okunseri, C. (2008). Socio-demographics of adult orthodontic visits in the United States. *Am J Orthod Dentofacial Orthop, 133*(4), 489.e489-414. doi:10.1016/j.ajodo.2007.08.016

Wilcko, W. M., Wilcko, T., Bouquot, J. E., & Ferguson, D. J. (2001). Rapid orthodontics with alveolar reshaping: two case reports of decrowding. *Int J Periodontics Restorative Dent, 21*(1), 9-19.

Willekens, I., Buls, N., Lahoutte, T., Baeyens, L., Vanhove, C., Caveliers, V., . . . de Mey, J. (2010). Evaluation of the radiation dose in micro-CT with optimization of the scan protocol. *Contrast Media Mol Imaging, 5*(4), 201-207. doi:10.1002/cmmi.394

Wishney, M. (2017). Potential risks of orthodontic therapy: a critical review and conceptual framework. *Aust Dent J, 62 Suppl 1*, 86-96. doi:10.1111/adj.12486

Xu, Y., Zhao, T., Xu, W., & Ding, Y. (2013). Periodontal microstructure change and tooth movement pattern under different force magnitudes in ovariectomized rats: an in-vivo microcomputed tomography study. *Am J Orthod Dentofacial Orthop, 143*(6), 828-836. doi:10.1016/j.ajodo.2013.01.020

Yaffe, A., Fine, N., & Binderman, I. (1994). Regional accelerated phenomenon in the mandible following mucoperiosteal flap surgery. *J Periodontol, 65*(1), 79-83. doi:10.1902/jop.1994.65.1.79

Yi, J., Xiao, J., Li, Y., Li, X., & Zhao, Z. (2017). Efficacy of piezocision on accelerating orthodontic tooth movement: A systematic review. *The Angle Orthodontist, 87*(4), 491-498. doi:10.2319/01191-751.1

Yip, G., Schneider, P., & Roberts, E. W. (2004). Micro-computed tomography: high resolution imaging of bone and implants in three dimensions. *Seminars in Orthodontics, 10*(2), 174-187. doi:10.1053/j.sodo.2004.01.005

Yu, J.-H., Huang, H.-L., Liu, C.-F., Wu, J., Li, Y.-F., Tsai, M.-T., & Hsu, J.-T. (2016). Does Orthodontic Treatment Affect the Alveolar Bone Density? *Medicine, 95*(10), e3080. doi:10.1097/MD.0000000000003080

Zhuang, L., Bai, Y., & Meng, X. (2011). Three-dimensional morphology of root and alveolar trabecular bone during tooth movement using micro-computed tomography. *Angle Orthod, 81*(3), 420-425. doi:10.2319/071910-418.1

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Presentation at scientific conference:

No.	Title of presentation	Conference	Awards
1	Spread of mandibular trabeculae pattern in relation to different micro-osteoperforation (MOP) intervals for accelerating orthodontic tooth movement	25th Malaysian Association of Orthodontists International Scientific Conference and Trade Exhibition. 27th – 29th April 2019, Kuala Lumpur	Winner for Oral Presentation (please refer to Appendix A and B)

Publications:

No.	Title of publication	Journal	Status
1	Osseous Evidence Behind Micro-Osteoperforation Technique In Accelerating Orthodontic Tooth Movement Towards Reducing Treatment Duration	American Journal of Orthodontics and Dentofacial Orthopedics	First revision