Application of Mechanical Vibration to Enhance the Stability After Orthodontic Treatment - A Micro-CT Study

Nicolas Branshaw
Marquette University

Recommended Citation
https://epublications.marquette.edu/theses_open/486
APPLICATION OF MECHANICAL VIBRATION TO ENHANCE THE STABILITY AFTER ORTHODONTIC TREATMENT - A MICRO-CT STUDY

By:
Nicolas R. Branshaw, M.A. D.M.D.

A Thesis submitted to the Faculty of the Graduate School, Marquette University, in Partial Fulfillment of the Requirements for the Degree of Master of Science

Milwaukee, WI
August 2018
Abstract

APPLICATION OF MECHANICAL VIBRATION TO ENHANCE THE STABILITY AFTER ORTHODONTIC TREATMENT - A MICRO-CT STUDY

Nicolas R. Branshaw, M.A. D.M.D.
Marquette University, 2018

Introduction: Mechanical vibration (MV) possesses anti-resorptive properties that can possibly enhance the stability of the dentition during retention, lessening the potential for relapse of teeth to their initial positions. In this project, we established a mouse model and investigated the effects of MV on bone modeling during orthodontic retention.

Materials and Methods: Thirty-two 14-week-old inbred strain C57BL/6 male mice were randomly assigned into four groups: 1) Control (N=6); 2) Mechanical Vibration (MV) (N =6); 3) Orthodontic Retention (OR) (N=9); and 4) Orthodontic Retention and Mechanical Vibration (ORMV) (N=11). All mice in OR and ORMV groups received approximately 10g of orthodontic force by a coil spring to move maxillary right 1st molar mesially for 10 days, followed by a retention period of 2 weeks. In MV and ORMV groups, the mice received MV (60 Hz, 0.3g) for 5min/day on the maxillary right 1st molar throughout the retention period. Micro-focus computed tomography (micro-CT) was used to quantify new bone formation through bone volume fraction (BVF), crestal bone heights and intermolar distances post-orthodontic retention. For each of the parameters, one-way ANOVA was used to examine whether there is a statistically significant difference among the 4 experimental groups, with Tukey comparison being used to determine the significant difference between each 2 groups (p < 0.05 is considered significant).

Results: In general, mechanical vibration produced significant changes of alveolar bone height and bone volume fraction among the experimental groups. For the alveolar bone height (mm) at distal buccal root, the order from least to greatest was MV (0.23±0.021) < Control (0.24±0.045) < ORMV (0.31±0.073) < OR (0.33±0.092). For the BVF (%) at furcation of M1, the order from least to greatest was OR (0.49±0.134) < ORMV (0.52±0.078) < Control (0.62±0.072) < MV (0.66±0.082). For the BVF (%) at interproximal between M1 and M2, the order from least to greatest was OR (0.49±0.115) < ORMV (0.49±0.115) < Control (0.69±0.051) < MV (0.74±0.028).

Conclusion: Mechanical vibration (60Hz, 0.3g, 5min/day) is able to increase the bone volume at furcation and interproximal and the crestal bone heights, indicating its potential clinical application to enhance the stability of orthodontic treatment.
ACKNOWLEDGEMENTS

Nicolas R. Branshaw, M.A. D.M.D.

I am truly thankful for all the amazing people who helped with this project. I would like to express my gratitude and appreciation to my committee members, Dr. Dawei Liu, Dr. Jeffery Toth, Drs. Ahuja Bhoomika and Nimeri Ghada for their assistance and support through the presentation of this thesis.

I would like to submit a special thanks to the architect of this groundbreaking research, Dr. Dawei Liu. He has been a mentor and a friend working with unrelenting passion to further my orthodontic knowledge, all the while simultaneously balancing clinical and administrative duties.

Additionally, I would like to thank Dr. Andrei Taut, for his time, effort and expertise. His academic wisdom and experience is well beyond that of his colleagues and I know he will be asset to any institution or practice that he joins. I am excited for what he will bring to the orthodontic field of study and the joy he will bring his patients. I would also like to thank Andrei for helping us to partner with Michelle Lynch at the University of Michigan’s micro-CT Core. We are truly thankful for all of Michelle’s time and effort scanning, uploading and troubleshooting the micro CT scans that contributed the bulk of our results in this study. Without her efforts, none of this project would have been possible.

Thank you to Drs. Ahuja Bhoomika and Nimeri Ghada for their clinical leadership and project support. Their experience and dedication to the program has made them invaluable to Marquette University Orthodontic Program.

Lastly, I would like to thank my wife, family and friends for their support and unyielding encouragement, as I would not have been able to succeed without them. Their partake in this journey has brought me much joy, making the time and effort, meaningful and fulfilling.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.............................................................................................................i  
TABLE OF CONTENTS.............................................................................................................ii  
LIST OF TABLES......................................................................................................................iii  
LIST OF FIGURES....................................................................................................................iv  

## CHAPTER

I. INTRODUCTION................................................................................................................... 1  

II. LITERATURE REVIEW............................................................................................................. 3  
    A. Biology of Orthodontic Tooth Movement................................................................. 3  
    B. Accelerated Tooth Movement...................................................................................... 5  
    C. Mechanisms of Cellular Differentiation Under Mechanical Vibration............. 6  
    D. Retention....................................................................................................................... 6  
    E. Health Benefits of Mechanical Vibration................................................................. 8  
    F. Mechanical Vibration Effects on Bone....................................................................... 9  
    G. Mouse Calvaria Anatomy.......................................................................................... 10  
    H. Micro-Computed Tomography.................................................................................... 11  

III. MATERIALS AND METHODS............................................................................................ 12  
    A. Experimental Design.................................................................................................... 12  
    B. Appliance Placement.................................................................................................... 12  
    C. Mechanical Vibration.................................................................................................. 14  
    D. Retention of Orthodontic Tooth Movement............................................................. 15
F. Clinical Implications................................................................. 41

VI. CONCLUSION................................................................................. 42

BIBLIOGRAPHY.................................................................................. 43
LIST OF TABLES

Table 1. Repeat Measurements for Intraclass Correlation Coefficient (ICC).................23
Table 2. Statistical Calculation of the Intraclass Correlation Coefficient (ICC)........23
Table 3. Descriptives: Distance from CEJ to Crestal Bone at Distal Buccal Root.........26
Table 4. ANOVA: Distance from CEJ to Crestal Bone at Distal Buccal Root............27
Table 5. Comparisons: Distance from CEJ to Crestal Bone at Distal Buccal Root.......27
Table 6. Descriptives: Distance from CEJ to Crestal Bone at Mesial Root.............29
Table 7. ANOVA: Distance from CEJ to Crestal Bone at Mesial Root.....................29
Table 8. Comparisons: Distance from CEJ to Crestal Bone at Mesial Root..............30
Table 9. Descriptives for Mean Furcation BVF..............................................32
Table 10. ANOVA Analysis for Mean Furcation BVF........................................33
Table 11. Multiple Comparisons for Mean Furcation BVF..................................33
Table 12. Descriptives for Mean Interproximal BVF.......................................35
Table 13. ANOVA Analysis for Mean Interproximal BVF.................................35
Table 14. Multiple Comparisons for Mean Interproximal BVF............................36
LIST OF FIGURES

Figure 1. Mechanism of Orthodontic Tooth Movement.................................4
Figure 2. Examples of Orthodontic Retainers..............................................8
Figure 3. Orthodontic Vibration Device – AcceleDent®.................................10
Figure 4. Anatomy of a Mouse Calvaria......................................................10
Figure 5. Micro-surgery Tools.................................................................13
Figure 6. Experimental Set-up for Mice.....................................................14
Figure 7. Manipulation of Mice During Experiment....................................15
Figure 8. Micro-computed Tomography System SCANCO Medical AG........17
Figure 9. Hard Tissue Micro-CT images....................................................18
Figure 10. Definition of Furcation Region of Interest (ROI).........................20
Figure 11. Definition of the Interproximal ROI.........................................21
Figure 12. Measurement of Tooth Movement............................................24
Figure 13. Determination of Height of Crestal Bone to CEJ of the Distal Buccal Root...26
Figure 14. Bar Graph: Distance from Crestal Bone to CEJ of the Distal Buccal Root....28
Figure 15. Determination of Height of Crestal Bone to CEJ of the Mesial Root........28
Figure 16. Bar Graph: Distance from Crestal Bone to CEJ of the Mesial Root.........30
Figure 17. Determination of BVF of the 1st molar ROI................................32
Figure 18. Bar Graph for the mean BVF of the Furcation ROI’s....................34
Figure 19. Determination of BVF of the Interproximal ROI..........................34
Figure 20. Bar Graph for the mean BVF at Interproximal ROI’s....................36
Chapter I

Introduction

Orthodontic forces produce physiologic changes within the dento-alveolar complex. The response of the dentition to these forces necessitates the development of a new equilibrium within the oral cavity. Orthodontic appliances, oral habits, hard and soft tissues create a complex matrix of forces that contributes to the movement of teeth within the periodontium. The goal of orthodontic treatment is to produce a stable, esthetic and functional occlusion. While the final stage of orthodontic therapy is orthodontic retention which aims to maintain the teeth in their corrected positions after the active orthodontic tooth movement.

Retainers (fixed or removable) are routinely prescribed for long periods of use as teeth have a strong tendency to return to their initial positions after active orthodontic tooth movement (Maltha et al., 2017; Horowitz et al., 1969). Patients often struggle with compliance of the prescribed retention protocols during retention (Kacer et al., 2010), often leading to orthodontic relapse. Orthodontic retention can even compromise oral hygiene possibly causing discomfort to the patients (Artun, 1984; Artun et al., 1987; Pandis et al., 2007). The importance of increased post-orthodontic stability is significant as the reduction of potential relapse and accordingly shortened retention time provides meaningful benefits to the patients.

Various new technological and surgical innovations have been proposed to accelerate orthodontic treatment based on the chemical and cellular responses they elicit
during treatment (Nimeri et al., 2013; Almpani et al., 2016). Few of those treatments have been proposed to enhance the stability of orthodontic treatment during retention. More specifically, mechanical vibration produces anabolic and catabolic effects on the periodontal support structures of the dentition (Thompson et al., 2014; Nishimora et al., 2008), demonstrating a promising and yet minimally invasive adjunct to treatment that could improve the stability of the dentition post-orthodontic treatment.

Bone volume and periodontal reorganization are significant factors that affect orthodontic tooth movement and retention (Yu et al., 2016). Studies have shown that mechanical vibration has anabolic effects on bone mass and its architecture both in orthopedics and dentistry (Thompson et al., 2014; Alikhani et al., 2018; Nishimora et al., 2008). However, no knowledge is known about the effects of mechanical vibration on the modeling/formation of alveolar bone after active orthodontic tooth movement. In this project, we generated a mouse retention model to investigate whether mechanical vibration promotes stability of tooth position after active orthodontic movement by determining differential alveolar bone formation between experimental groups.

Increased bone volume fraction (BVF) and decreased crestal alveolar bone heights from CEJ are indicators for increased periodontal stability. This study utilized micro-focused computed tomography (micro-CT) to measure BVF and alveolar bone heights of mice calvarias for the 4 experimental groups, i.e. control, mechanical vibration, orthodontic retention and orthodontic retention with mechanical vibration.
Chapter II

Literature Review

Biology of Orthodontic Tooth Movement

Consistent force application on the dentition produces pressure and tension areas within the periodontal ligaments, as depicted in Figure 1, producing chemical and electrical signals that initiate resorption and apposition of bone within the alveolus. This biologic response provides the mechanism of tooth movement within the alveolar bone and the foundation of orthodontic tooth movement. Further understanding of this mechanism through research and clinical experience has established various theories on how to enhance the efficiency of tooth movement and the stability during retention.

Biologic electricity is created initially during tooth movement through the piezoelectric effect. Orthodontic piezoelectricity is a flow of electrons through the alveolus derived from the alteration of the crystalline lattice structures within periodontal ligament and bone, following force application. The flow of electrons demonstrates a quick rate of decay, though the piezoelectric flow initiates again as the force is released from the tooth and the crystal lattice is able to return to its original position. (Profit et al. 2013) The piezoelectric signals affect orthopedic and dento-alveolar structures producing adaptations to stresses placed on the skeletal structures. Research has shown that mechanical vibration amplifies the piezoelectric signals during orthodontic tooth movement, accelerating the metabolic and anabolic processes of the dento-alveolar complex (Shapiro et al., 1979).

The pressure-tension theory is generally accepted as the foundational theory of orthodontic tooth movement. Orthodontic force generates pressure and tension within the
periodontal ligament initiating blood flow restrictions and chemical messengers that signal cellular differentiation, enabling tooth movement within periodontium. (Profit et al. 2013). Compression or pressure within the periodontal ligament restricts blood flow and initiates the release of chemical messengers that further stimulate an osteoclastic and mononuclear cellular response to metabolize bone structures (Raisz, 1999). Conversely, tension within the periodontal ligament initiates the release of chemical messengers to signal bone formation through osteoblastic activity and bone apposition. The deposition and apposition of alveolar bone structures occurs through a mechanism that ensures equivalent amounts of bone are replaced as to maintain the integrity of periodontium (Hill, 1998).

**Figure 1.** Mechanisms of Orthodontic Tooth Movement. a) Histologic cross-section view of tooth root under orthodontic force, demonstrating pressure and tension of the periodontal ligament (Melsen et al., 2006). b) Histological slide showing the pressure side during orthodontic movement. From left to right: tooth structure (dentin and cementum), disorganized periodontal fibers and bone (Melsen et al., 2006). c) Sagittal diagram of pressure and tension areas within the periodontal ligament after application of orthodontic force to the tooth.
**Accelerated Tooth Movement**

Anabolic and catabolic processes in alveolar bone modeling are crucial to orthodontic tooth movement and the methods to incite these processes can accelerate orthodontic tooth movement. It was discovered early in the 19th century that bone modeling and remodeling were accelerated during wound healing. A pioneering American oral surgeon, Hullihan, had experimented with corticotomies to accelerate tooth movement with this improved bone remodeling. Corticotomies and wound incitation though effective at accelerating orthodontic tooth movement entail high cost of surgery, possible iatrogenic effect, potential infections and a low but still present morbidity that must be taken into account for an elective procedure (Proffit et al., 2013).

Other physical processes that have been proposed to accelerate orthodontic tooth movement are application of light to alveolus, therapeutic ultrasound to the periodontium and mechanical vibration (Proffit et al., 2013; Almpani et al., 2016). Light therapy is believed to induce vasodilation for increased blood flow, excite cellular enzymes and release pro-inflammatory mediators that will accelerate wound healing, although evidence is of low quality in support of light therapy in accelerated tooth movement (Sonnesson et al., 2017). Therapeutic ultrasound has been shown to increase blood flow to treatment areas and it is theorized that the increased blood flow will accelerate tooth movement (Proffit et al., 2013). Promising research has suggested that mechanical vibration incites anabolic and metabolic pathways to accelerate orthodontic tooth movement (Alikhani et al., 2018), although presently very little is known about the effectiveness of many of these less invasive wound healing adjunctive procedures on orthodontic retention.
Mechanisms of Cellular Differentiation Under Mechanical Vibration

Studies have suggested that differentiation of mesenchymal stem cells toward the osteogenic pathway occurs more frequently under mechanical vibration. Up regulation of bone morphogenetic proteins (BMP) and RUNX2 proteins, as well as phosphorylation of Smad1, ERK1/2 and p38 MAPK under low intensity vibration significantly increase induction of stem cell differentiation to osteoblast and/or chondroblasts (Suzuki et al., 2009; Angle SR et al., 2011; Ikeda et al., 2016). Furthermore, evidence has shown that low intensity mechanical vibration down regulates NF-kappaB ligand (RANKL) and up regulates osteoprotegerin (OPG), establishing an environment that increases bone regeneration (Maddi et al., 2006), as well as inhibited osteoclast formation through DC-STAMP (Kulkarni et al., 2012). Overall, in vitro studies imply the potential use of mechanical vibration in producing a denser and more stable alveolus post orthodontic tooth movement.

Retention

Retention is the final phase of orthodontic treatment that aims to maintain the occlusion in an improved esthetic and functional position after active orthodontic treatment. Inflammation within the periodontal tissues that occurs during active treatment initiates catabolic processes within the dento-alveolar complex (Alikhani et al., 2018); altering bone densities (Yu et al., 2016), enlarging PDL spaces, and activating metabolic signals in an attempt to establish a new equilibrium (Ikeda et al., 2016). The physiologic condition of the periodontium at the start of the retention can be considered a recovery from physiologic changes that have occurred during treatment. Utilization of fixed and removable retainers, as illustrated in Figure 2, are necessary to stabilize the dento-
alveolar complex after orthodontic therapy, as teeth have tendency to relapse towards their initial position (Horowitz et al., 1969).

Relapses prevention and occurrence happens through complex biologic processes that include skeletal growth, rearrangement of periodontal ligaments, bone metabolism, and soft tissue adaptations (Maltha et al., 2017). Clinicians have sought to find ways to increase orthodontic stability post active treatment. Researchers have suggested both physical and chemical adjuncts to improve orthodontic stability during retention. The invasiveness and cost of physical surgical procedures, such as cortocotomies and conventional circumferential supracrestal fiberotomy (CSF), have prohibited many clinicians from studying or exploiting their potential benefits in improving orthodontic retention (Jahanbin et al., 2014). Pharmacologic agents, many of which are naturally occurring proteins, could help decrease relapse potential of the dentition (Hudson et al., 2012; Kim et al., 1999; Hassan et al., 2010). Even with FDA approval, storage and use of pharmacologic agents in retention would be cost prohibitive for minimal if any benefit to the patient.

Less invasive physical adjunctive devices, such as low frequency ultrasound, Low Level Laser Therapy (LLLT), and low frequency mechanical vibration have been proposed to accelerate orthodontic tooth movement, however little is known about their effects on orthodontic retention (Sonnesson et al, 2017; Jing et al., 2017; Proffit et al., 2013). Studies have shown that low frequency ultrasound increases production of Bone Morphogneic Protein, a protein necessary for bone apposition, however no clinical evidence has been provided on its ability to improve orthodontic retention (Suzuki et al.,
LLLT has some evidence of translation demonstrating prevention of relapse improves with laser therapy in the short term (Jahanbin et al., 2014).

Low frequency mechanical vibration has demonstrated promising anabolic effects on craniofacial tissues in the absence of inflammation, such as that caused in orthodontic tooth movement (Xie et al., 2006; Alikhani et al., 2018). Low frequency mechanical vibration could have a significant effect on improving alveolar bone density during the retention phase of orthodontic treatment (Alikhani et al., 2018). Although research has supported a promising foundation and adjunctive devices are already approved by FDA for clinical use, few studies have been conducted to assess the effects of mechanical vibration on orthodontic retention.

Figure 2. Examples of orthodontic retainers. a) bonded fixed retainer (Image from Dear Doctor Inc.) and b) Hawley retainer (Image from Dolphin Aquarium software).

**Health Benefits of Mechanical Vibration**

Vibration research has shown evidence of providing generalized health benefits. Orthopedic vibrational research has demonstrated improved bone density in patients with osteoporosis (Li et al., 2006; Xie et al., 2006). Studies in exercise science have shown low magnitude vibration reduces muscle soreness by improving blood flow to effected
areas (Veqar et al., 2014). Additionally, vibration has shown to improve flexibility, balance, muscle strength and coordination (Veqar et al., 2014; Uhm et al., 2018; Almpani et al., 2016).

**Mechanical Vibration Effects on Bone**

Studies have shown that mechanical vibration has anabolic and catabolic effects on bone mass and architecture, both in orthopedics and dentistry (Thompson et al., 2014; Nishimora et al., 2008). Active orthodontic therapy initiates a physiologic inflammation, which in the presence of mechanical vibration activates catabolic functions (Alikhani et al., 2018). This increased metabolic turnover is the mechanism that allows for accelerated orthodontic tooth movement while under mechanical vibration (Pavlin et al., 2015). Whereas in the absence of inflammation mechanical vibration signals anabolic processes that some researchers have suggested could play a significant role in stabilizing the dentition during orthodontic retention (Alkahani et al., 2018).

Studies have suggested that ranges of frequencies with adjunctive mechanical vibration devices are effective at accelerating orthodontic tooth movement. Established companies, such as AcceleDent®, retail adjunctive mechanical vibration devices (Figure 3) to clinicians, stating that these devices accelerate orthodontic tooth movement. The U.S. Food and Drug Administration (FDA) has approved AcceleDent® as a Class II medical device proven to move teeth up to 50% faster (AcceleDent website). AcceleDent®’s low frequency mechanical vibration occurs at 30 Hz with .3g of acceleration. Recent translational research has shown significant improvement in orthodontic tooth movement seen at 60 Hz and 120 Hz, but without significant difference between 60 Hz and 120 Hz (Alkahani et al., 2018). Future clinical research should be conducted at a higher frequency beyond what is currently being clinically prescribed to patients.
Figure 3. Orthodontic vibration device - AcceleDent®. This device provides mechanical vibration at a frequency of 30 Hz with .3g of acceleration, used 20 min per day.

Mouse Skull Anatomy

The average dimensions of mouse calvaria are approximately length 25 mm anterior-posterior, width 10 mm left-right, and height 10 mm superior-inferior (Kawakami et al., 2008). In this study, the experimental area of interest was 5 mm anterior to the right maxillary 1st molar to the distal of right maxillary 3rd molar as depicted in Figure 4. Calvaria were resected superior to the anterior ethmoidal foramen and posterior to the tympanic bulla to reduce bone volume for CT scans (Figure 4).

Figure 4. Anatomy of a mouse calvaria. a) Anatomy of the left sagittal view of a mouse calvaria highlighting the side contralateral to the Region of Interest that will be scanned by micro-CT to measure variations in bone height and bone volume fraction. b) Inferior horizontal view of the mouse calvaria, highlighting to the experimental Region of Interest.
Micro-Computed Tomography

Computed Tomography in dentistry has enabled clinicians to better visualize constructs of alveolar bone, tooth morphology, accessory canals, impaction and resorption of the dentition. This 3-dimensional rendering of the hard tissues within dental facial complex is quickly becoming the standard of care in many clinical situations. The use of this technology has only been limited by the cost and the level of radiation produced. However, the costs have decreased and limited fields of view/scan speeds have reduced exposure significantly. From a research perspective, micro-CT provides significant experimental value for hard tissue density and volumes of alveolar bone (Ohiomoba et al., 2017; Park et al., 2009).
Chapter III

Materials and Methods

Experimental Design

In this project we investigated the effects of mechanical vibration on orthodontic tooth retention. Thirty-two 14-week-old inbred strain C57BL/6 male mice were randomly assigned into four groups:

1) Control (N=6)
2) Mechanical Vibration (MV) group (N =6)
3) Orthodontic Retention (OR) group (N=9)
4) Orthodontic Retention and Mechanical vibration (ORMV) group (N=11)

All the 20 mice undergoing orthodontic treatment received approximately 10 g of force through a coil spring to move maxillary right 1st molar anteriorly for 10 days, followed by a retention period of 2 weeks. In MV and ORMV groups, we applied mechanical vibration (60 Hz, 0.3g) for 5min/day on the maxillary right 1st molar throughout the retention period while the control and OR groups were treated under the same condition but without vibration.

Appliance Placement

In accordance with the Recommended Best Practices for Mouse Anesthesia & Analgesia designed by Marquette University’s Office of Research and Compliance (ORC), mice were placed under general anesthesia by injection with Ketamine (100 mg/kg) and Xylazine (10 mg/kg) prior to appliance delivery. Once fully anesthetized a .030 in. stainless steel (SS) wire was used to fabricate a custom mouth prop (Figure 6a)
to retract the buccal soft tissue while separating maxillary and mandibular incisors for an unobstructed surgical field. Titanium micro needle holders for ophthalmic surgery (Figure 5) were used to pass separate 0.009 inch. SS ligature wire beneath the contact of right maxillary 1st and 2nd molars and between the maxillary central incisors. Each ligature was then ligated to a custom fabricated nickel – titanium mini-spring (G&H Company) approximately 3.5 mm in length. During ligation the custom spring was activated to produce an orthodontic force intended to mesialize the right maxillary 1st molar. A self-etching primer and flowable composite were used to prevent dislodgement of the central incisor ligation, as no height of contour is present to retain the ligation. 1st molar ligation was tied beneath the height of contour with no composite attachment, as accidentally bonding 1st and 2nd molars together would significantly affect results.

Figure 5. Micro-surgery tools. The titanium micro needle holders for ophthalmic surgery were used during appliance placement to provide a minimally invasive approach to appliance placement (https://www.ebay.com/i/323201898564?chn=ps).
Figure 6. Experimental setting-up for mice. a) a mouse under inhalation anesthesia, with the custom mouth prop in place and b) an illustration of the orthodontic appliance utilized in our study (Image from d’Appuzzo 2013). (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3655650/)

Mechanical Vibration

Post induction of anesthesia through isoflurane inhalation, mice was propped on a platform as seen in Figure 7b. Unilateral Mechanical vibration was conducted through an electromechanical actuator held in place by a custom apparatus as demonstrated in Figure 7b. Custom software (LabView) was designed to communicate with the electromechanical actuator to produce specific frequencies of vibration. Vibration was conducted at 0.3g (acceleration), 20 micrometers of micro-displacement (vertical), and 60 Hz frequency, for 5min/day. Inhalation of isoflurane was used for daily sedation as research has shown injectable anesthesia affect metabolic processes when used daily, which could skew experimental results.
Retention of orthodontic tooth movement

To halt active orthodontic treatment, Flow Tain®, a light curable composite material was injected into the threads of the spring coils and cured. The deactivated spring then acted as a fixed retainer during the retention phase in this study. The retention phase was conducted for 14 days. Fukui et al. (2003) suggests that a significant amount of periodontal reorganization occurs following orthodontic tooth movement of 8 days in rats. When extrapolated to mice, 14 days would allow for significant bone recovery and periodontal recovery to occur.

Euthanasia

Euthanasia is defined as the termination of animals by stimulating rapid unconsciousness followed by death without producing pain or distress. Pain is derived through nerve impulses that reach the brain. Consequently, an unconscious animal is unable to feel pain. Respiratory and cardiac arrest preceded by an expedited unconsciousness is necessary for a suitable euthanasia method.
Euthanasia of mice within this animal model is consistent with the 2007 Report of the AVMA Guidelines on Euthanasia. Guidelines recommend inhalation of CO$_2$ to depress the cerebral cortex, preventing pain and distress, while rapid unconsciousness and death occur. Mice were placed in a sealed chamber with a CO$_2$ flow rate of 4 liters per minute, until animals appeared unconscious (approximately 1 min). Flow rates of CO$_2$ were then adjusted to 10 liters per minute for four minutes causing asphyxiation. Followed by cervical dislocation and decapitation to ensure death.

**Tissue Preparation**

The mandible and all soft tissues were physically separated from the mouse calvaria. Additionally, the cranium was removed from the maxilla to minimize the micro-CT scan volume. Calvaria were washed 3 times in PBS. Fixation in formalin for 24 hours then into 70% ethanol till examination.

**Micro-Computed Tomography**

Distance measurements of molar tooth movement, alveolar bone height and bone volume fraction (BVF) were analyzed using micro-computed tomography (SCANCO Medical AG, Bruttisellen, Switzerland) (Figure 8). Peak Kilo voltage was set to 55 kVp with an intensity of 145 JA. Voxel size was set to 8 µm.
Figure 8. The micro-computed tomography system (SCANCO Medical AG, Bruttisellen, Switzerland).

Bone Parameters

Bone Volume Fraction (BVF) was analyzed on micro-computed tomographies (SCANCO Medical AG, Bruttisellen, Switzerland). For quantitative analyses, the micro-CT images were cropped to a region of interest (ROI) that was limited to the furcation of the maxillary right 1st molar. Scanning parameters followed guidelines set by Yadav et al. (2015). Samples were scanned individually within 16 mm chamber under high resolution in a liquid medium. Peak voltage was set to 55 kVp with an intensity of 145 JA. Voxel size was set to 8 µm to provide the necessary resolution for accurate measurement of the bone volume fraction within the alveolus. Threshold of bone within each scan was established through histogram analysis and then averaged over all scans to the equivalent 6555 units, which was used to specify bone in all scans.
Figure 9. 3D rendering images of Micro-CT scans to depicting the effects of OTM and MV on the maxillary 1st molar region labeled as “S”. a) Control group; b) Mechanical Vibration (MV) group; c) Orthodontic Retention (OR) group; and d) Orthodontic Retention and Mechanical Vibration (ORMV).

Tooth movement measurement

Measurements of the 1st molar orthodontic tooth movement were made through micro-focus computed tomography. Micro-CT slices were oriented and aligned along the occlusal plane and the central fossa. Distances measured through the sagittal slice at the greatest height of contour on the distal of maxillary right 1st to the mesial height of contour of the maxillary right 2nd molar, as demonstrated between blue and green arrows in Figure 12.
**Differences in Crestal Bone Height**

Differences in crestal bone height were measured to establish the effectiveness of vibration in alveolar bone regeneration post orthodontic movement. Sagittal and frontal planes of the micro-CT scans were aligned separately through pulpal chambers of distobuccal and mesial roots of the maxillary right 1st molar. Distances were measured through the sagittal plane from the CEJ to the height of crestal bone at each root respectively. Measurements for each group are depicted in Figures 16 and 17.

**Furcation Region of Interest Analysis**

Bone volume fraction of the right 1st molar was measured to establish the effectiveness of vibration on alveolar bone regeneration. The horizontal plane of the micro-CT was reoriented to be leveled with the apex of all three roots of maxillary right 1st molar. Points were established at each root within alternating slices of the horizontal plane (Figure 10a) and then interpolated to generate a three-dimensional Region of Interest (ROI) (Figure 10b). For the furcation ROI, points were established in three locations, i.e. the most distal point of the mesial root, the most mesial point of the distal buccal root, and the most mesial buccal point of the distal lingual root. These points are demonstrated in Figure 10.
**Figure 10.** Definition of furcation region of interest (ROI). a) Horizontal plane demonstrating points to generate the furcation ROI. From left to rights roots around the established points on most mesial point of distal buccal root, most mesial buccal point on distal lingual root and most distal point on distal buccal root. b) ROI was generated through points placed in a) throughout all horizontal slices.

**Interproximal Region of Interest (ROI) Analysis**

Bone volume fractions (BVF) between the upper right 1st and 2nd molars were measured to establish the effectiveness of vibration on alveolar bone modeling/formation. The horizontal plane of the micro-CT was oriented mesio-distally to level with the occlusal plane of the maxillary right 1st and 2nd molars, then oriented buccal-lingually to the apex of the mesial lingual and mesial buccal roots of the maxillary right 2nd molar. The interproximal ROI between the 1st and 2nd molars was developed through 4 points at each of the 4 roots present within each slice of the horizontal plane. The points were located at the most mesial portion of the mesial buccal and mesial lingual roots of the maxillary 2nd molar, then at the most distal point of the maxillary lingual root and the most distal lingual point of the distal buccal root of the maxillary 1st molar. The points were then interpolated between slices to generate the interproximal Region of Interest, as seen in **Figure 11**.
Figure 11. Definition of the interproximal ROI. a) Horizontal plane demonstrating points to generate the furcation ROI. Points were located at the most mesial portion of the mesial buccal and mesial lingual roots of the maxillary 2nd molar, then at the most distal point of the maxillary lingual root and the most distal lingual point of the distal buccal root of the maxillary 1st molar. b) ROI was generated through points placed in a) throughout all horizontal slices.

Intra-Examiner Reliability

Variability between scans is inherently present when the examiner selects points of interest within each scan. To examine the variability present within the examiner (N.B.), we conduct an intra-examiner reliability test – represented by intraclass Correlation Coefficient (ICC). Significance was measured in terms of $p$ value $<.05$ and the measurements were correlated to the ICC with 1 being ideal. Two-way mixed effects model where people effects are random and measures effects are fixed. The estimator is the same, whether the interaction effect is present or not. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.
Statistical Analysis

Descriptive statistics, ANOVA analysis, and multiple comparison Tukey analysis were used to examine the distribution and differences of BVF and crestal bone heights among the 4 experimental groups ($p < 0.05$ is considered significant).
Chapter IV

Results

Intra-Examiner Reliability

Variability between measurements is inherently present when the examiner selects points of interest within each scan. To examine the variability present within the examiner (N.B.), we conducted an intra-examiner reliability test – represented by Intraclass Correlation Coefficient (ICC). Measurements for all the 5 variables in one scan in this study were repeated three times every two days apart. The correlation of all the measures of 3 times demonstrated high significance (p < 0.000), indicating that all the measurements conducted at the 3 times were very close to each other, as ICC were 0.999 (1 is ideal). Results depicted in Table 1 and Table 2.

Table 1. Repeated measurements for the Intraclass Correlation Coefficient. Repeat measurements for one scan three times every two days for heights of distal buccal and mesial root crestal bone to CEJ respectively as well as for the furcation BVF of the ROI.

<table>
<thead>
<tr>
<th>TIME</th>
<th>Total Volume (mm^3) furcation</th>
<th>Bone Volume (mm^3) furcation</th>
<th>BVF Interproximal</th>
<th>Distal buccal root Bone-CEJ</th>
<th>Mesial Buccal Root Bone-CEJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.076</td>
<td>0.0422</td>
<td>0.5559</td>
<td>0.23</td>
<td>0.45</td>
</tr>
<tr>
<td>T2</td>
<td>0.0764</td>
<td>0.0441</td>
<td>0.5767</td>
<td>0.25</td>
<td>0.43</td>
</tr>
<tr>
<td>T3</td>
<td>0.0749</td>
<td>0.0437</td>
<td>0.5837</td>
<td>0.24</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 2: Statistical calculation of the Intraclass Correlation Coefficient.

<table>
<thead>
<tr>
<th></th>
<th>Intraclass Correlationb</th>
<th>95% Confidence Interval</th>
<th>F Test with True Value 0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Single Measures</td>
<td>.998</td>
<td>.990</td>
<td>1.000</td>
</tr>
<tr>
<td>Average Measures</td>
<td>.999</td>
<td>.997</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Note: As shown, ICC = 0.998 (p < 0.000) indicating that points selected demonstrated a high rate of consistency.
**Intermolar distance**

Due to appliance dislodgement in all but one mouse in the OR group, most intermolar distances displayed at the end of experiment were not measurable. A single experimental mouse that displayed 1st molar orthodontic movement (Figure 12b), and the intermolar distances were measured through micro-CT analysis. The sagittal plane was aligned through the central fossa and distances were measured from the height of contour of the 1st and 2nd molars, seen in Figure 12a. This mouse displayed orthodontic tooth movement of .19 mm as measured through micro-CT analysis.

![Figure 12](image.png)

**Figure 12.** Measurement of tooth movement. a) Sagittal plane of the micro-CT scan aligned through the central fossa, measuring intermolar distance from the distal of maxillary 1st molar to the mesial of maxillary 2nd molar at the heights of contour. b) The calvaria dissection demonstrating 1st molar mesial displacement under orthodontic force.

**Tooth to Crestal Bone Relationship**

Bar graphs (Figure 13) represent the experimental group means and standard deviations for the height of crestal bone to cemental enamel junction (CEJ) around the distal buccal and mesial roots of the maxillary 1st molar. Micro-CT examples of each experimental group distances measured around the distal buccal root and mesial root are
depicted in Figures 13 and 15. Variations in heights of crestal bone were measured indirectly through their relationship to the CEJ of the maxillary 1st molar, indicating an inverse relationship between data collected and crestal bone heights. In other words, larger values from the data (Figure 14 and Tables 3, 4, 5) equate to lower crestal bone heights and a reduction of crestal bone.

Mean values of the data collected from the CEJ of the distal buccal root to crestal bone (See Figure 14 and Tables 3, 4, 5) strongly correlates with BVF of interproximal and furcation ROI’s, indicating that crestal bone increased with mechanical vibration (MV vs. Control) and crestal bone heights decrease with orthodontic tooth movement (ORMV vs. OR). Whereas mean values of the distance from the CEJ of the mesial root to crestal bone demonstrate congruent data for non-retention groups, though retention groups where incongruent with previous findings, as seen in Figure 16 and Tables 6, 7, 8. The mesial crestal bone heights of retention group demonstrated greater crestal bone heights than that of the retention and vibration group. None of the data collected from our experimental groups was statistically significant possibly due to large variation and small sample size.
Figure 13. Determination of the height of crestal bone (blue arrow) to CEJ (green arrow) around the distal buccal root of the maxillary 1st molar in each experimental group: a). Control group. b). MV group. c). OR group d). ORMV group.

Table 3. Descriptive results of the distances (mm) from CEJ to crestal bone for the distal buccal root.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>.238333</td>
<td>.0449073</td>
<td>.018333</td>
<td>.191206</td>
<td>.285461</td>
<td>.1700</td>
</tr>
<tr>
<td>MV</td>
<td>6</td>
<td>.230000</td>
<td>.0209762</td>
<td>.0085635</td>
<td>.207987</td>
<td>.252013</td>
<td>.1900</td>
</tr>
<tr>
<td>OR</td>
<td>9</td>
<td>.330000</td>
<td>.0916515</td>
<td>.0305505</td>
<td>.259550</td>
<td>.400450</td>
<td>.2300</td>
</tr>
<tr>
<td>OR+MV</td>
<td>11</td>
<td>.305455</td>
<td>.0728510</td>
<td>.0219654</td>
<td>.256513</td>
<td>.354397</td>
<td>.2000</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>.285625</td>
<td>.0775819</td>
<td>.0137147</td>
<td>.257654</td>
<td>.313596</td>
<td>.1700</td>
</tr>
</tbody>
</table>
Table 4. ANOVA analysis of the distances (mm) from CEJ to crestal bone for the distal buccal root.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups (Combined)</td>
<td>.054</td>
<td>3</td>
<td>.018</td>
<td>3.804</td>
<td>.021</td>
</tr>
<tr>
<td>Linear Term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unweighted</td>
<td>.035</td>
<td>1</td>
<td>.035</td>
<td>7.390</td>
<td>.011</td>
</tr>
<tr>
<td>Weighted</td>
<td>.032</td>
<td>1</td>
<td>.032</td>
<td>6.706</td>
<td>.015</td>
</tr>
<tr>
<td>Deviation</td>
<td>.022</td>
<td>2</td>
<td>.011</td>
<td>2.354</td>
<td>.114</td>
</tr>
<tr>
<td>Within Groups</td>
<td>.133</td>
<td>28</td>
<td>.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>.187</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Multiple (Tukey) comparisons between each two groups for the distances from CEJ to crestal bone for the distal buccal root.

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MV</td>
<td>.0083333</td>
<td>.0397247</td>
<td>.997</td>
<td>-.100127</td>
<td>.116794</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td>-.0916667</td>
<td>.0362635</td>
<td>.077</td>
<td>-.190677</td>
<td>.007344</td>
<td></td>
</tr>
<tr>
<td>OR+MV</td>
<td></td>
<td>-.0671212</td>
<td>.0349199</td>
<td>.242</td>
<td>-.162463</td>
<td>.028221</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td>Control</td>
<td>-.0083333</td>
<td>.0397247</td>
<td>.997</td>
<td>-.116794</td>
<td>.100127</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td>-.1000000</td>
<td>.0362635</td>
<td>.047</td>
<td>-.199011</td>
<td>-.009989</td>
<td></td>
</tr>
<tr>
<td>OR+MV</td>
<td></td>
<td>-.0754545</td>
<td>.0349199</td>
<td>.159</td>
<td>-.170797</td>
<td>.019888</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>Control</td>
<td>.0916667</td>
<td>.0362635</td>
<td>.077</td>
<td>-.007344</td>
<td>.190677</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td></td>
<td>.1000000</td>
<td>.0362635</td>
<td>.047</td>
<td>.000989</td>
<td>.199011</td>
<td></td>
</tr>
<tr>
<td>OR+MV</td>
<td></td>
<td>.0245455</td>
<td>.0309256</td>
<td>.857</td>
<td>-.059891</td>
<td>.108982</td>
<td></td>
</tr>
<tr>
<td>OR+MV</td>
<td>Control</td>
<td>.0671212</td>
<td>.0349199</td>
<td>.242</td>
<td>-.028221</td>
<td>.162463</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td></td>
<td>.0754545</td>
<td>.0349199</td>
<td>.159</td>
<td>-.019888</td>
<td>.170797</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td>.0245455</td>
<td>.0309256</td>
<td>.857</td>
<td>-.108982</td>
<td>.059891</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 14.** Bar graph of the mean ± SD of the 4 experimental groups for the height of crestal bone to cemental enamel junction (CEJ) around the distal buccal root of the maxillary 1st molar.

**Figure 15.** Determination of the height of crestal bone (blue arrow) to CEJ (green arrow) around the mesial root of the maxillary 1st molar in each experimental group: a). Control group. b). MV group. c). OR group d). ORMV group.
Table 6. Descriptive results of the distances (mm) from the CEJ at the mesial to the crestal bone.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>.496667</td>
<td>.0608824</td>
<td>.0248551</td>
<td>.432775</td>
<td>.560559</td>
<td>.4200</td>
<td>.5800</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td>6</td>
<td>.438333</td>
<td>.0608002</td>
<td>.0248216</td>
<td>.374527</td>
<td>.502139</td>
<td>.3300</td>
<td>.5000</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>9</td>
<td>.517778</td>
<td>.1153015</td>
<td>.0384338</td>
<td>.429149</td>
<td>.606406</td>
<td>.3400</td>
<td>.7000</td>
<td></td>
</tr>
<tr>
<td>OR+MV</td>
<td>11</td>
<td>.540000</td>
<td>.0804984</td>
<td>.0242712</td>
<td>.485920</td>
<td>.594080</td>
<td>.4300</td>
<td>.6900</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>.506563</td>
<td>.0898245</td>
<td>.0158789</td>
<td>.474177</td>
<td>.538948</td>
<td>.3300</td>
<td>.7000</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. ANOVA Analysis of the distances (mm) from the CEJ at the mesial root to the crestal bone.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>(Combined)</td>
<td>3</td>
<td>.014</td>
<td>1.881</td>
<td>.156</td>
</tr>
<tr>
<td>Linear Term</td>
<td>Unweighted</td>
<td>1</td>
<td>.017</td>
<td>2.273</td>
<td>.143</td>
</tr>
<tr>
<td></td>
<td>Weighted</td>
<td>1</td>
<td>.020</td>
<td>2.736</td>
<td>.109</td>
</tr>
<tr>
<td></td>
<td>Deviation</td>
<td>2</td>
<td>.011</td>
<td>1.453</td>
<td>.251</td>
</tr>
<tr>
<td>Within Groups</td>
<td>.208</td>
<td>28</td>
<td>.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>.250</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8. Multiple (Tukey) comparisons between each two groups for the distances from the CEJ at the mesial root to the crestal bone

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval Lower Bound</th>
<th>95% Confidence Interval Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MV</td>
<td>.0583333</td>
<td>.0497819</td>
<td>.649</td>
<td>-.077587</td>
<td>.194254</td>
</tr>
<tr>
<td>OR</td>
<td>- .0211111</td>
<td>.0454445</td>
<td>.966</td>
<td></td>
<td>-.145189</td>
<td>.102966</td>
</tr>
<tr>
<td>OR+MV</td>
<td>- .0433333</td>
<td>.0437608</td>
<td>.756</td>
<td></td>
<td>-.162814</td>
<td>.076147</td>
</tr>
<tr>
<td>MV</td>
<td>Control</td>
<td>- .0583333</td>
<td>.0497819</td>
<td>.649</td>
<td>-.194254</td>
<td>.077587</td>
</tr>
<tr>
<td>OR</td>
<td>- .0794444</td>
<td>.0454445</td>
<td>.319</td>
<td></td>
<td>-.203522</td>
<td>.044633</td>
</tr>
<tr>
<td>OR+MV</td>
<td>- .1016667</td>
<td>.0437608</td>
<td>.117</td>
<td></td>
<td>-.221147</td>
<td>.017814</td>
</tr>
<tr>
<td>OR</td>
<td>Control</td>
<td>.0211111</td>
<td>.0454445</td>
<td>.966</td>
<td>-.102966</td>
<td>.145189</td>
</tr>
<tr>
<td>MV</td>
<td>.0794444</td>
<td>.0454445</td>
<td>.319</td>
<td></td>
<td>-.044633</td>
<td>.203522</td>
</tr>
<tr>
<td>OR+MV</td>
<td>- .0222222</td>
<td>.0387552</td>
<td>.939</td>
<td></td>
<td>-.128036</td>
<td>.083591</td>
</tr>
<tr>
<td>OR+MV</td>
<td>Control</td>
<td>.0433333</td>
<td>.0437608</td>
<td>.756</td>
<td>-.076147</td>
<td>.162814</td>
</tr>
<tr>
<td>MV</td>
<td>.1016667</td>
<td>.0437608</td>
<td>.117</td>
<td></td>
<td>-.017814</td>
<td>.221147</td>
</tr>
<tr>
<td>OR</td>
<td>.0222222</td>
<td>.0387552</td>
<td>.939</td>
<td></td>
<td>-.083591</td>
<td>.128036</td>
</tr>
</tbody>
</table>

Figure 16. Bar graph of the mean ± SD of the 4 experimental groups for the height of crestal bone to cemental enamel junction (CEJ) around the mesial root of the maxillary 1st molar.
**Bone Volume Fraction (BVF)**

The stability of tooth positions after orthodontic tooth movement depends mainly on the quantity of the newly formed alveolar bone around the orthodontically repositioned teeth during retention. The quantity of alveolar bone in the experimental groups thus represents dental stability. On the Micro-CT scans of the mouse calvaria, Bone Volume Fraction (BVF) was measured in two locations, the furcation of the 1st molar (Figure 17) and the interproximal bone between the 1st and 2nd molars (Figure 18). BVF was calculated from two ROI’s extrapolated from the methods discussed above. Results from ROI’s in both the furcation and interproximal molar region provided congruent BVF data. Mean values from both furcation (Figure 18 and Tables 9, 10, 11) and interproximal (Figure 19 and Tables 12, 13, 14) ROIs indicated that vibration produced an increase in BVF and orthodontic tooth movement decreased BVF. Averages of BVF indicate that vibration could increase the alveolar bone concentrations post orthodontic movement, though results were not statistically significant (Tables 11 and 14). Interproximal BVF did demonstrate a significant decrease between Control and OR groups, indicating that orthodontic tooth movement decreased BVF (Table 14).
Figure 17. Determination of BVF of the 1st molar furcation ROI in each of the experimental group. a). Control group. b). MV group. c). OR group d). ORMV group.

Table 9. Descriptive statistics for mean furcation BVF (%) of experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean (mm³/mm³)</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
<th>Minim um</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>.614917</td>
<td>.0721790</td>
<td>.0294669</td>
<td>.539169</td>
<td>.690664</td>
<td>.5468</td>
<td>.7289</td>
</tr>
<tr>
<td>MV</td>
<td>6</td>
<td>.656133</td>
<td>.0821844</td>
<td>.0335516</td>
<td>.569886</td>
<td>.742381</td>
<td>.5509</td>
<td>.7779</td>
</tr>
<tr>
<td>OR</td>
<td>9</td>
<td>.492178</td>
<td>.1340975</td>
<td>.0446992</td>
<td>.389101</td>
<td>.595254</td>
<td>.2780</td>
<td>.7226</td>
</tr>
<tr>
<td>OR+MV</td>
<td>11</td>
<td>.518800</td>
<td>.0774853</td>
<td>.0233627</td>
<td>.466745</td>
<td>.570855</td>
<td>.3775</td>
<td>.6896</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>.555084</td>
<td>.1131028</td>
<td>.0199939</td>
<td>.514306</td>
<td>.595862</td>
<td>.2780</td>
<td>.7779</td>
</tr>
</tbody>
</table>
**Table 10.** ANOVA Analysis of mean furcation BVF (%) of experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Groups</strong></td>
<td>(Combined)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear Term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unweighted</td>
<td>.079</td>
<td>1</td>
<td>.079</td>
<td>8.367</td>
<td>.007</td>
</tr>
<tr>
<td>Weighted</td>
<td>.075</td>
<td>1</td>
<td>.075</td>
<td>7.990</td>
<td>.009</td>
</tr>
<tr>
<td>Deviation</td>
<td>.058</td>
<td>2</td>
<td>.029</td>
<td>3.057</td>
<td>.063</td>
</tr>
<tr>
<td><strong>Within Groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.264</td>
<td>28</td>
<td>.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>.397</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 11.** Multiple comparisons for mean furcation BVF (%) of experimental groups. The mean difference is significant at the 0.05 level.

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MV</td>
<td>-.0412167</td>
<td>.0560312</td>
<td>.882</td>
<td>-.194199 -.111766</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>.1227389</td>
<td>.0511492</td>
<td>.100</td>
<td>-.016914 .262392</td>
</tr>
<tr>
<td></td>
<td>OR+MV</td>
<td>.0961167</td>
<td>.0492541</td>
<td>.230</td>
<td>-.038362 .230596</td>
</tr>
<tr>
<td>MV</td>
<td>Control</td>
<td>.0412167</td>
<td>.0560312</td>
<td>.882</td>
<td>-.111766 .194199</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>.1639556*</td>
<td>.0511492</td>
<td>.017</td>
<td>-.024302 .303609</td>
</tr>
<tr>
<td></td>
<td>OR+MV</td>
<td>.1373333*</td>
<td>.0492541</td>
<td>.044</td>
<td>.002854 .271812</td>
</tr>
<tr>
<td>OR</td>
<td>Control</td>
<td>-.1227389</td>
<td>.0511492</td>
<td>.100</td>
<td>-.262392 .016914</td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>-.1639556*</td>
<td>.0511492</td>
<td>.017</td>
<td>-.303609 -.024302</td>
</tr>
<tr>
<td></td>
<td>OR+MV</td>
<td>-.0266222</td>
<td>.0436202</td>
<td>.928</td>
<td>-.145719 .092474</td>
</tr>
<tr>
<td>OR+MV</td>
<td>Control</td>
<td>-.0961167</td>
<td>.0492541</td>
<td>.230</td>
<td>-.230596 .038362</td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>-.1373333*</td>
<td>.0492541</td>
<td>.044</td>
<td>-.271812 -.002854</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>.0266222</td>
<td>.0436202</td>
<td>.928</td>
<td>-.092474 .145719</td>
</tr>
</tbody>
</table>
Figure 18. Bar graph of the experimental groups mean values and variability for furcation BVF. (* P < .05)

Figure 19. Determination of BVF of Interproximal 1st and 2nd molar ROI in each of the experimental group. a). Control group. b). MV group. c). OR group d). ORMV group.
Table 12. Descriptive statistics for mean interproximal BVF (%) of experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean (mm³/mm³)</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>.685150</td>
<td>.0509205</td>
<td>.0207882</td>
<td>.631712</td>
<td>.738588</td>
<td>.6058</td>
</tr>
<tr>
<td>MV</td>
<td>6</td>
<td>.737133</td>
<td>.0276634</td>
<td>.0112935</td>
<td>.708102</td>
<td>.766164</td>
<td>.7057</td>
</tr>
<tr>
<td>OR</td>
<td>9</td>
<td>.433044</td>
<td>.1489026</td>
<td>.0496342</td>
<td>.318588</td>
<td>.547501</td>
<td>.2355</td>
</tr>
<tr>
<td>OR+MV</td>
<td>11</td>
<td>.491273</td>
<td>.1149293</td>
<td>.0346525</td>
<td>.414062</td>
<td>.568483</td>
<td>.2375</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>.557347</td>
<td>.1611730</td>
<td>.0284916</td>
<td>.499238</td>
<td>.615456</td>
<td>.2355</td>
</tr>
</tbody>
</table>

Table 13. ANOVA Analysis of mean interproximal BVF (%) of experimental groups.

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Combined)</td>
<td>.479</td>
<td>3</td>
<td>.160</td>
<td>13.704</td>
</tr>
<tr>
<td>Linear Term</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unweighted</td>
<td>.302</td>
<td>1</td>
<td>.302</td>
<td>25.936</td>
</tr>
<tr>
<td>Weighted</td>
<td>.283</td>
<td>1</td>
<td>.283</td>
<td>24.245</td>
</tr>
<tr>
<td>Deviation</td>
<td>.197</td>
<td>2</td>
<td>.098</td>
<td>8.433</td>
</tr>
<tr>
<td>Within Groups</td>
<td>.326</td>
<td>28</td>
<td>.012</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>.805</td>
<td>31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 14. Multiple comparisons for mean furcation BVF (%) of experimental groups. The mean difference is significant at the 0.05 level.

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval Lower Bound</th>
<th>95% Confidence Interval Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MV</td>
<td>-.0519833</td>
<td>.0623216</td>
<td>.838</td>
<td>-1.148757</td>
<td>-1.222141</td>
</tr>
<tr>
<td>OR</td>
<td>MV</td>
<td>.2521056*</td>
<td>.0568915</td>
<td>.001</td>
<td>.096774</td>
<td>.407437</td>
</tr>
<tr>
<td>OR+MV</td>
<td>MV</td>
<td>.1938773*</td>
<td>.0547837</td>
<td>.007</td>
<td>-.044301</td>
<td>.343454</td>
</tr>
<tr>
<td>MV</td>
<td>OR</td>
<td>.0519833</td>
<td>.0623216</td>
<td>.838</td>
<td>-.118174</td>
<td>.222141</td>
</tr>
<tr>
<td>OR</td>
<td>OR+MV</td>
<td>.3040889*</td>
<td>.0568915</td>
<td>.000</td>
<td>.148757</td>
<td>.459421</td>
</tr>
<tr>
<td>OR+MV</td>
<td>OR+MV</td>
<td>.2458606*</td>
<td>.0547837</td>
<td>.001</td>
<td>.096284</td>
<td>.395437</td>
</tr>
<tr>
<td>OR</td>
<td>Control</td>
<td>-.2521056*</td>
<td>.0568915</td>
<td>.001</td>
<td>-1.096774</td>
<td>-1.407437</td>
</tr>
<tr>
<td>OR</td>
<td>MV</td>
<td>-.3040889*</td>
<td>.0568915</td>
<td>.000</td>
<td>-.459421</td>
<td>-1.148757</td>
</tr>
<tr>
<td>OR+MV</td>
<td>OR+MV</td>
<td>-.0582283</td>
<td>.0485173</td>
<td>.632</td>
<td>-.190696</td>
<td>.074239</td>
</tr>
<tr>
<td>OR</td>
<td>MV</td>
<td>-.1938773*</td>
<td>.0547837</td>
<td>.007</td>
<td>-.343454</td>
<td>-.044301</td>
</tr>
<tr>
<td>OR+MV</td>
<td>OR</td>
<td>-.2458606*</td>
<td>.0547837</td>
<td>.001</td>
<td>-.395437</td>
<td>-.096284</td>
</tr>
<tr>
<td>OR</td>
<td>MV</td>
<td>.0582283</td>
<td>.0485173</td>
<td>.632</td>
<td>-.074239</td>
<td>.190696</td>
</tr>
</tbody>
</table>

Figure 20. Bar graph of the experimental group mean values for interproximal BVF (%), as well as the range of values for each group. (** P < .01)
Chapter V

Discussion

Animal Species

Previous studies used rabbits (Al-Sayagh et al., 2014), rats (Alikhani et al., 2018) and mice (Yadav et al., 2015). In this study we used thirty-two 14-week-old inbred strain C57BL/6 male mice to provide greater reliability through increased sample sizes. Size and cost of other species often limits studies to small samples sizes, increasing chances for statistical error and decreasing the significance. While many researchers avoid the size limitations of the dentition and oral cavity in mice, our research team found that the right tools enabled us to overcome these limitations.

Experiment Design

In this project, we investigated the effect of mechanical vibration on orthodontic tooth retention. Thirty-two 14-week-old inbred strain C57BL/6 male mice were randomly assigned into four groups:

1) Control (N=6)
2) Mechanical Vibration (MV) group (N =6)
3) Orthodontic Retention (OR) group (N=9)
4) Orthodontic Retention and Mechanical vibration (ORMV) group (N=11)

Twenty mice in OR and ORMV groups received approximately 10g of force by a coil spring to move maxillary right 1st molar anteriorly for 10 days, followed by a retention period of 2 weeks. In MV and FMV groups we applied mechanical vibration (60 Hz,
0.3g) for 5 min/day on the maxillary right 1st molar throughout the retention period while the control group were treated under the same condition without vibration.

**Orthodontic Tooth Movement (OTM)**

Orthodontic tooth movement (OTM) as measured by the distance between the 1st and 2nd molars at the height of contour (See Figure 12) was only found in one experimental mouse at a distance of .19 mm of space by the end of the 2 weeks retention simply because the coil spring was remained to the end. OTM though was believed to have occurred in the rest of experimental mice as the majority of mice lost the coil appliances between days 3 and 5 at the 1st molar attachment. During appliance placement a 0.009” wire was passed beneath the contact point between the 1st and 2nd molars, then tied around the 1st molar. Similar animal models with rats indicated that the wire tied beneath the height of contour would be enough to hold appliance placement at the molar attachment. Dislocation of the appliance from the 1st molar attachment is believed to have occurred as orthodontic tooth movement opened space between 1st and 2nd molars, the wire was able to slip over the height of contour and through the separated contact point halting orthodontic tooth movement. Indicating at least 0.01” of space was created through orthodontic tooth movement for the 1st molar wire to be passed through the contact during the first few days of the orthodontic tooth movement phase.

Considerations for future appliance placement may consider placing bonding material over the wire at the 1st molar attachment. Though significant effort should be put in place to prevent bonding materials from bonding the 1st and 2nd molars together, as this mistake would significantly alter results.
Mechanical Vibration

Current clinical devices use low frequency mechanical vibration at 30 Hz with 0.3g of acceleration to enhance OTM; and recent translational research has shown significant improvement in OTM seen at 60 Hz and 120 Hz (Alkahani et al., 2018). The improvement of tooth movement at higher frequencies in comparison to what is being clinically prescribed indicates the need for future studies at higher frequencies. This study has expanded on initial mechanical vibration retention studies conducted at lower frequencies of 30 Hz (Yadav et al., 2015), in an effort to more effectively demonstrate the affects of higher frequency vibration on retention. In addition, this retention model created a fixed retention period during vibration, allowing for bone regeneration to occur and bypass the greatest period of potential relapse.

Our study demonstrated that the BVFs at furcation and interproximal regions as well as crestal bone height means were consistent with the current evidence of mechanical vibration improving bone volume regeneration (Alikhani et al., 2016). Therefore, the increased bone regeneration could have a positive effect on the stability of post orthodontic treatment, although results from this study were not significant. Mesial crestal bone heights for both retention groups demonstrated incongruent data in comparison to other consistent changes in this study can be explained that the alveolar bone mesial to the 1st molar underwent significant resorption allowing tooth to move orthodontically thus its reversal to bone formation may take longer time which is not revealed in the time window of this study. Though a large variability and small sample sizes created significant overlap between both experimental retention groups. Increased distance from mesial crestal bone to CEJ for retention and vibration group may indicate
that longer period of retention and vibration is necessary for the bone that only
experiences resorption. None of the data between experimental groups was significant.

**Orthodontic Retention (OR)**

Previous retention studies (Yadav et al., 2015) have expanded on relapse potential
under mechanical vibration, although these studies lacked a realistic study of retention
stability. Relapse potential is greatest during the initial period post removal of
orthodontic forces. Yadav et al. (2015) allowed for relapse to occur with no period of
retention and no initial alveolar bone regeneration to begin. Anabolic processes necessary
for bone regeneration from mechanical vibration occur over several days post removal of
orthodontic forces. A stabilization period should be established while mechanical
vibration is occurring through the retention period. This procedure may negate the
greatest initial relapse and allow the accelerated cellular metabolic processes of
mechanical vibration to initiate.

**Limitations**

Several imitations exist in this study, including sample size and appliance
dislocation, resulted in the lack of significance in experimental results. Larger samples
sizes of experimental groups could provide more consistent results, providing greater
significance to the data collected. The appliances delivered in the experimental groups
were dislocated from molar region, due to a lack of retention provided by the height of
contour of the distal portion of the 1st molar. Ligation of the 1st molar region may require
future application of composite bonding to prevent dislocation of the appliance, though
great care should be maintained not to bond 1st and 2nd molars together. Mesial crestal
bone only experienced resorption and results indicated that retention only demonstrated greater crestal bone heights. Longer retention periods may be necessary to provide improved results consistent with past studies.

**Clinical Implications**

Current clinical device AcceleDent utilizes mechanical vibration at 30 Hz with 0.3g of acceleration to enhance orthodontic tooth movement. Recent translational research has shown significant improvement in orthodontic tooth movement seen at 60 Hz and 120 Hz (Alikhani et al., 2018). Indicating two major implications for clinical application. FDA approved devices for accelerated orthodontics are readily available for study on the effects of mechanical vibration during orthodontic retention. Additionally, our findings of BVF of furcation and interproximal regions as well as crestal bone height indicate further study of mechanical vibration at higher frequencies may provide improved results for clinical devices.
Chapter VI

Conclusion

Although not statistically significant between OR and ORMV groups, the mean BVFs (both M1 furcation and interproximal between M1 and M2) and interproximal crestal bone heights demonstrate a positive correlation between mechanical vibration and alveolar bone regeneration for both OR and ORMV groups. These findings implicate the use of mechanical vibration during the retention phase of orthodontic treatment to improve the stability of tooth positions after orthodontic movement.
BIBLIOGRAPHY


Kitaura, Hideki, Keisuke Kimura, Masahiko Ishida, Haruki Sugisawa, Haruka Kohara, Masako Yoshimatsu, and Teruko Takano-Yamamoto. “Effect of Cytokines on Osteoclast Formation and Bone Resorption during Mechanical Force Loading of the Periodontal


