

Application of Mechanical Vibration to Modulate Orthodontic Tooth Movement (OTM) in Mice

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APPLICATION OF MECHANICAL VIBRATION TO MODULATE ORTHODONTIC
TOOTH MOVEMENT (OTM) IN MICE
-A PILOT MICRO CT STUDY

by

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A thesis submitted to the Faculty of the Graduate School, Marquette University,
in Partial fulfillment of the Requirement for the Degree of Master of Science

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ABSTRACT

APPLICATION OF MECHANICAL VIBRATION TO MODULATE ORTHODONTIC TOOTH MOVEMENT (OTM) IN MICE

-A PILOT MICRO CT STUDY

Andrew Rummel

Marquette University, 2010

Purpose: The purpose of this study was to use micro CT to evaluate the effect of mechanical vibration on orthodontic tooth movement (OTM) in mice. **Materials and Methods:** Thirty-six mice (C57BL/6, 20 weeks old, male) were randomly divided into four groups: spring/vibration (SV), spring only (S), vibration only (V), control (C). A nickel-titanium (NiTi) spring was fabricated and inserted bilaterally between the maxillary 1st molars, delivering an initial force of 20 grams. From day 0, mechanical vibration (4Hz frequency, 20micron displacement, 5 min per session duration) was applied to the left maxillary 1st molar every three days for 3 weeks (21 days). Following the completion of the experiment, animals were euthanized and the harvested maxillae were evaluated by micro CT analysis. Various variables (5 on coronal view and 6 on axial view) were defined and measured on the micro CT images. One way ANOVA with Dunnett's post hoc was employed to determine the statistical significance of differences between the vibrated and non-vibrated sides, and among the treatment groups (p value set at 0.05). **Results:** Significant orthodontic tooth movement was observed in the spring and spring/vibration groups versus the control and vibration alone groups (p<0.05). Differences between spring and spring/vibration groups as targeted were not identified statistically however an overall tendency was observed demonstrating that the vibrated side had increased OTM when compared to its contra-lateral non-vibrated side in the spring/vibration group. **Conclusion:** Micro CT can be used to evaluate orthodontic tooth movement in mice, which however requires improvement to further study the effect of mechanical vibration on orthodontic tooth movement.

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CHAPTER I: INTRODUCTION

The specialty of orthodontics is very rewarding practice both for the patient and practitioner. Orthodontists are able to change smiles, improve self esteem, and improve function. However, there are drawbacks associated with orthodontics such as root resorption, increased susceptibility to oral hygiene issues, compliance, and treatment duration. Of importance to orthodontists in reference to these issues is treatment time. The less time fixed appliances are used, there is less likelihood these factors could cause problems to the patient's health.¹ The use of mechanical vibration to modulate orthodontic tooth movement might be a strategy towards decreasing treatment time.

CHAPTER II: LITERATURE REVIEW

Mouse-Experimental Model

At the present time, the laboratory mouse has become a central tool for skeletal studies, mainly because of the extensive use of genetic manipulations in this species.² The ability to manipulate the genetics of the mouse model provides many opportunities for researchers in different disciplines. Examples of genetic manipulations include transgenic and knockout species. Knockout mice have one or more genes that have been turned off by a targeted mutation.³ By turning off particular genes, it may be possible to determine the function of the targeted gene. Mice are currently the most related laboratory animal species to humans, where knockouts can be easily produced.³ A transgenic mouse contains additional, artificially-introduced genetic material in every cell.³ This often confers a gain of function, for example, the mouse may produce a new protein, but a loss of function may occur if the integrated DNA interrupts another gene.³ A transgenic mouse is a very useful system for studying mammalian gene function and regulation because analysis is carried out on the whole organism. Until 2003, researchers were unable to manipulate the rat's genetic code to produce knockout specimens.⁴ However, given the method that was used, the genes may only be altered at random. The vast majority of information and ease of creating knockout mice may continue to entice researchers to use the mouse model.

Mouse Anatomy

The inbred strain C57BL/6 mice have maxillary 1st, 2nd, and 3rd molars with two incisors (Figures 1, 2). No premolars or canines are present. Each molar has three roots present: mesio-buccal, disto-buccal, and palatal. The roots

of the 1st molar are more defined and separated when compared to the 3rd molar, where the roots appear to be more convergent.

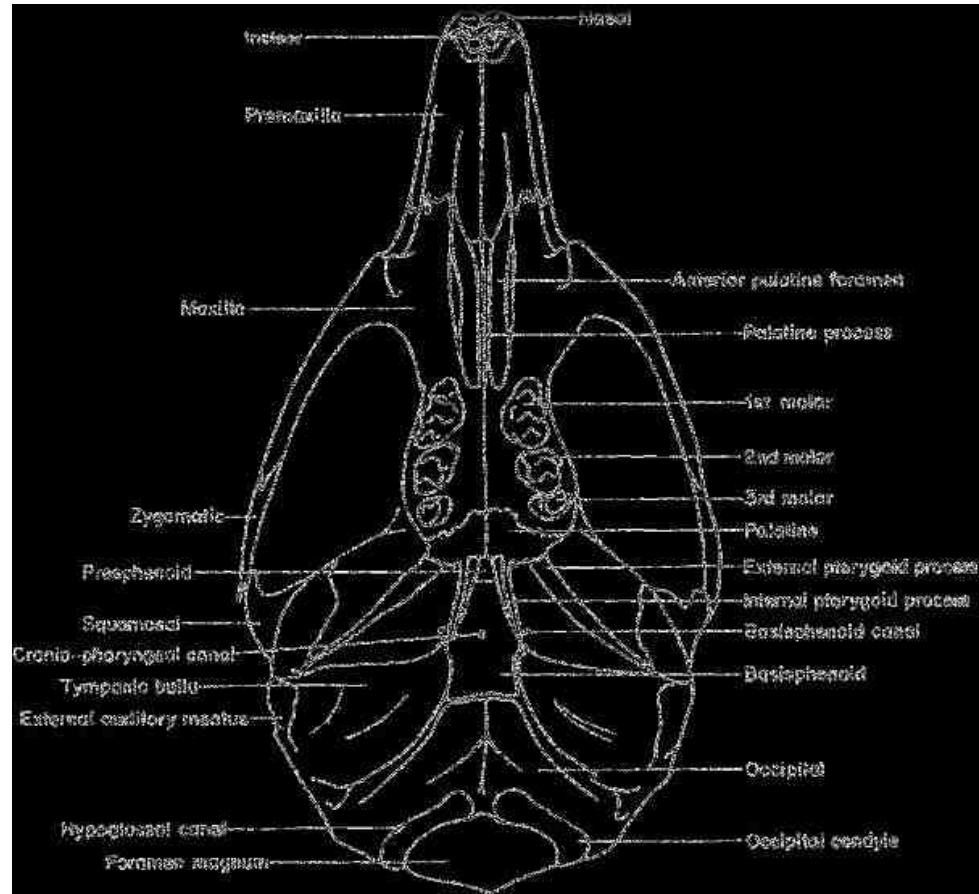


Figure 1: Diagram of mouse anatomy, axial view.⁵



Figure 2: Diagram of mouse anatomy, sagittal view.⁵

Age of Mice

Barnett et al, compared alveolar bone in young and aged mice.⁶ Young mice were 12 weeks old, while aged mice were 94 weeks old.⁶ In the current study we looked at 20 week old mice, comparing our study to the previous studies we could consider our mice to be grouped as adult. In regards to tooth movement in young or aged mice, there is very little information in the literature. However, it has been shown in rats who are 6 weeks old, that in the initial phase of tooth movement the rate was faster in the young rats than in aged rats (9-12 months old).⁷ Although once tooth movement reached the linear phase, the rate of tooth movement was the same in both groups.⁷

Bone

There are several different types of bone that are formed depending on the physiologic situation. The following list describes the various types of bone formed and cites situations where they may be present.

1. **Woven Bone:** weak, disorganized, and poorly mineralized. Plays a crucial role in wound healing by rapidly filling osseous defects, providing initial continuity for fractures and osteotomy segments and strengthening a bone weakened by surgery or trauma.⁸ Orthodontic tooth movement results in rapid formation of relatively immature new bone, which is woven bone.⁸ Woven bone can also be seen in the mid-palatal suture after rapid palatal expansion has taken place.
2. **Lamellar Bone:** strong, highly organized, well-mineralized, and makes up over 99% of the adult human skeleton.⁸ The full strength of lamellar bone that supports an orthodontically moved tooth is not achieved until about 1 year after completion of active treatment.⁸
3. **Composite Bone:** an osseous tissue formed by the deposition of lamellar bone within a woven bone lattice. Composite bone is an important intermediary type of bone in the physiologic response to orthodontic loading and it usually is the predominant osseous tissue for stabilization during the early process of retention or postoperative healing.⁸
4. **Bundle Bone:** a functional adaptation of lamellar structure to allow attachment of tendons and ligaments. Distinct layers of bundle bone are usually seen adjacent to the PDL along physiologic bone-forming surfaces.⁹

Mechanical Adaptation of Bone

Stress, in its simplest definition, occurs when a load is placed on bone which stretches the intermolecular bonds that resist with an elastic force.¹⁰ Strain occurs when a load is placed on bone and deforms it.¹⁰ Frost suggested that living bone may depend more on strain than stress to generate the signals that control its biological reactions to mechanical loads. Small strains would help to guide the remodeling and modeling phases of bone healing, however, excessive strains can usually prevent bony union.¹⁰ The naturally permissible strains might lie in the 100-2000 microstrain region, compared to bone's fracture strain which is in the 25,000 microstrain region.¹¹ Microstrain is a measurement defined as deformation per unit length (unit of strain, a microstrain is 10^{-6} of length change).¹² In 1987, Frost developed the mechanostat theory (Figure 3) to show the relationship between dynamic loading, strain, and bone response.

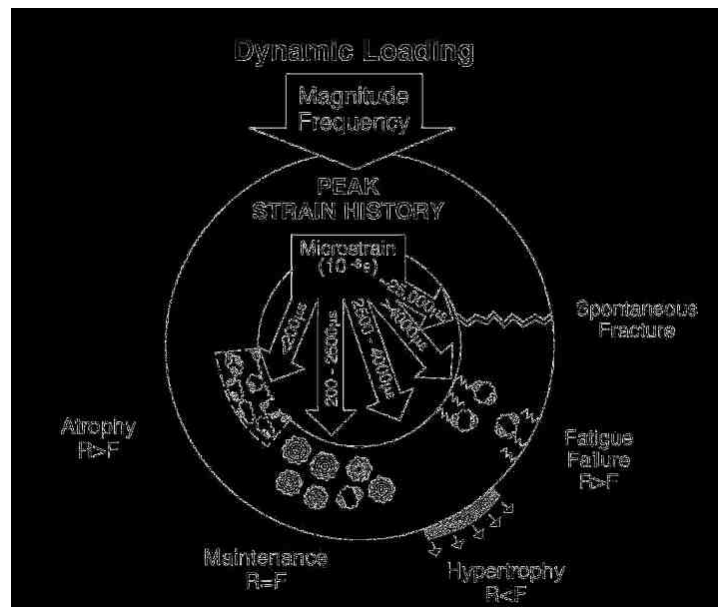


Figure 3: Diagram representing the ranges of microstrain and its result.¹²

The following is a set of interacting mechanical variables that may provide the signal for skeletal adaptation¹³

1. **Strain Magnitude:** The percentage change in length of a bone. If bone adapts to the magnitude of strain, then surface strains on bone should be proportional to cortical thickness. Burr suggested it may be more reasonable to expect a bone to adapt not to peak principal strain, but to the lower physiologic strain limit, the limit above which bone mass is retained rather than lost.
2. **Strain Distribution/Strain Direction:** The pattern of strain magnitude across a section of bone. The adaptive response of bone may be related to the way that strains are distributed within the bone. Lanyon (1987) proposed that the stimulus required to produce an adaptive response may change, depending upon the strain distribution.¹⁴ Lanyon proposed that the strain required to elicit an adaptive response may be lower if the manner of loading is different from the usual pattern of loading.¹⁴ Additionally, if the strain distribution is usual, the threshold to initiate an adaptive change may be higher, requiring a larger strain stimulus.¹⁴
3. **Stimulus Duration:** Total number of deformation cycles or the period of time over which they are applied. Burr mentioned that the overwhelming majority of evidence indicates that the duration of loading is a minor determinant of the magnitude of the adaptive remodeling response. A stimulus may be required to exceed the threshold for a relatively few cycles to elicit an adaptive response. Rubin and Lanyon demonstrated that 1800 cycles per day did not increase the remodeling more than 36 cycles per day did when using the same loading regimen.¹⁵

4. **Strain Rate:** The speed at which bone is deformed. Bone is considered to be a viscoelastic material. Repetitive loading is necessary to cause bone adaptation.
5. **Strain Frequency:** The number of deformation cycles/second or harmonics associated with these. Burr explained that frequency analysis of the strain signal generated during locomotion shows that there are significant strain frequency components up to about 50 Hz. McLeod and Rubin found that strain magnitudes normally associated with bone loss at 1 Hz cause significant new bone formation when applied at 15 Hz.¹⁶ They also found that high frequency components of strain (12-30Hz) predict a maximum osteogenic response for bone in the strain range of 2000-3000 microstrain, the range generally conceded to be the peak principal strain for most animals.¹⁶ Given this information it would be possible that bone is more or less responsive at different frequencies.
6. **Strain Polarity:** Tension, compression, or shear in particular planes of space. Burr described the different views of orthopedists and orthodontists, suggesting that the observation that cortical drift occurs is clear evidence that bone may either form or resorb on compressive and tensile surfaces. In general, orthodontists would argue that bone resorbs on the compressive side and apposition occurs on the tension side.
7. **Strain Energy Density:** The elastic energy stored in bone during deformation, or energy dissipated. The strain energy is equal to the product of the stress and strain, or the area under the stress/strain curve.

Remodeling/Modeling

Both trabecular and cortical bone grow, adapt, and turn over by two fundamentally distinct mechanisms: modeling and remodeling.¹⁷ Bone modeling involves independent sites of resorption and formation that change the form (shape and/or size) of a bone.¹⁷ However, bone remodeling is a specific coupled sequence of resorption and formation events to replace previously existing bone.¹⁷ Due to the difference of the two mechanisms, you are able to visually see modeling on x-rays, while remodeling is apparent at the microscopic level using histological analysis. According to Roberts, bone modeling is the dominant process in growth as well as in adaptation to applied loads such as headgear, rapid palatal expansion, and functional appliances.¹⁷ Both modeling and remodeling are controlled by an interaction of metabolic and mechanical signals.¹⁷ These signals include functional and applied loads, in addition parathyroid hormone and estrogen can play a role in the response of remodeling. Such as in pregnant women, orthodontic tooth movement is faster in contrast, patients with low bone turnover, such as hypothyroidism, see delayed appearance of resorption and formative activities.^{18,19}

Root Resorption

The loss of tooth substance has been referred to by many names; root absorption, external apical resorption, external resorption, and root resorption.²⁰ Factors associated with external apical root resorption include trauma, infection, and tooth movement. However, the effect of drugs, role of genetics, the immune system, patient age, patient gender and the patient's medical history have also been reviewed as factors that may be

associated with root resorption.^{21,22,23,24} In regards to resorption and tooth movement, many factors can play a role in the prediction and the amount of resorption that will take place.

1. **Tooth Vulnerability:** Most studies report that the upper lateral, upper central and lower incisors are the most frequently affected teeth. In addition, risk is also associated with the shape of the root, especially for those with blunt or pipette-shaped roots. Malmgren et al reported that traumatized teeth were not more prone to resorption than uninjured teeth.²⁵ However, Malmgren noted that traumatized teeth with signs of root resorption prior to treatment, may be more prone to root resorption during treatment.²⁵ Endodontically treated teeth have been suggested to be more resistant to resorption because of increased dentin hardness and density.²⁶
2. **Age:** Adult patients experience more resorption than younger patients.⁸ This was substantiated by the moderate number of cells and the fact that the fibrous tissue reacts more slowly and the turnover rate of collagen is in general slower in adults.⁸
3. **Orthodontic Appliances:** According to Linge and Linge, in regards to orthodontic appliances, highly significant risk factors were the use of rectangular archwires and Class II elastics.²⁷ Additionally, fixed appliances caused significantly more apical root resorption than removable appliances.²⁷
4. **Application of Force:** Studies have demonstrated that intermittent forces move teeth more physiologically than continuous forces.^{28,29} It is believed that this may allow the resorbed cementum to heal, which would lead to less resorption.²⁹
5. **Duration of Force:** Baumrind et al described that increased length of treatment time was positively associated with increased root resorption.³⁰

6. **Type of tooth movement:** As discussed by Graber, intrusion and torque are probably the most detrimental tooth movements.⁸ During these movements, radiographs must be taken, light forces used and frequent rest periods to provide an intermittent type force.

Resorption can be detected by the use of panoramic x-rays or periapical x-rays. Either form of diagnosis will show a blunting of the apical portion of the root in mild cases, to loss of more than one-third of the root in more severe cases. Detection of resorption radiographically is most obvious in the apical area; however, histologic investigation provides evidence that the same resorptive process also occurs on the side surface of the roots, which is difficult to find using routine radiographs.²⁰ Interestingly, no articles have reported tooth loss caused by severe apical root resorption associated with orthodontic therapy where no other form of trauma or infection was involved.²⁰ If apical root resorption is detected during treatment it is advisable to stop treatment for a period of time.

During orthodontic tooth movement many changes take place within the periodontium. The forces that are applied create strains in the tooth-supporting tissues that manifest almost immediately and can be roughly categorized as compressive and tensile.³¹ However, recently it was found that given the nonlinear behavior of the PDL, the classical zones of tension and compression could not be found.³² Rather than in distinct areas, the tension and compression zones are correlated to the alveolar bone morphology.³²

Resorption of the alveolar bone takes place on the compression side of the periodontal ligament, while apposition takes place on the tension side. When orthodontic forces are applied, if heavy, pain will develop almost immediately as the periodontal ligament is crushed.³³ The pain associated with the application of forces is related to the ischemic areas of the periodontal ligament. Initially, the force that is placed on the tooth, could be big enough to partially or totally occlude the blood supply to the periodontal ligament. During this occlusion of the periodontal ligament, necrosis ensues within the compressed area of the ligament.³³ Histologically, cells disappear in the areas of necrosis, and this avascular area becomes referred to as “hyalinized”.³³ During this period of hyalinization, tooth movement is ceased and a process of undermining resorption occurs.

Osteoclastogenesis, formation of osteoclasts, in orthodontic tooth movement is initiated by two related changes brought upon by the application of forces: tissue damage, with the subsequent production of inflammatory processes in the periodontal ligament; and deformation of the alveolar process.³¹ Osteoclasts appear within the adjacent bone marrow spaces and attack the underside of the bone immediately adjacent to the necrotic periodontal ligament area.³³ The delay that is experienced with this process results from the delay in cell differentiation in the necrotic areas and the amount of bone that has to be removed by undermining resorption. In regards to root resorption, the cells that resorb the necrotic tissue may also resorb the root surface indiscriminately.²⁰ This resorption process continues until no hyaline tissue is present and/or the force level decreases.³⁴ Reviewing reactivation schedules of force, Brezniak and Wassersten noted that the extent of root resorption was increased only when force reactivation was performed at the peak presence of osteoclast counts.³⁴

Repair of resorption has been observed as early as 3-5 weeks after the initiation of light orthodontic tooth movement.²⁰ Additionally, it is believed that the healing process of a resorption cavity starts when orthodontic force is discontinued or reduced to below a certain level.⁸ In regards to repair, secondary cementum is laid down in the resorption lacunae, which can be located in the apical, middle, or coronal portions of the root. Interestingly, Ownman-Moll et al demonstrated that human 1st premolars 8 weeks into retention had nearly 50% of the resorption lacunae with no repair or only partial coverage with healing cementum.³⁵

In 2006, Al-Qawasmi et al studied the genetic contributions associated with root resorption with the application of orthodontic forces in 8 different groups of inbred mice.³⁶ The study, which included the inbred strain C57BL/6 (the strain used for our study), applied 25 g of force using a coil spring from the maxillary first molar to the incisor for nine days.³⁶ Using histological analysis, it was found that the C57BL/6 inbred strain is actually fairly resistant to root resorption.³⁶

Mechanical Vibration

Currently in the literature there is limited information on using vibration to modulate orthodontic tooth movement. Nishimura et al looked at the effects of resonance vibration (application of vibration with continuously changing frequency onto teeth) on tooth movement and root resorption in rats.³⁷ Twelve male wistar rats were divided into 2 groups, .012 nickel titanium (NiTi) expansion spring with an initial force of 12.8 grams for 21 days.³⁷ Resonance frequency was performed on the occlusal surface of 1st molars for 8 minutes once a week, with an average frequency and displacement of 61.02 Hz and

.014 mm, respectively.³⁷ After 21 days, the amount of tooth movement in the vibration group was significantly greater (15%) than the control group.³⁷ There was no significant difference in the level of root resorption between the control group and vibration group.

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Studies in animals and humans have shown whole body vibration provides an anabolic potential to bone.^{38,39} Whole body vibration is thought to provide increased bone density to patient's with osteoporosis or for preventing bone loss in an aging or bed ridden patient.³⁸ Christiansen et al tested adult male C57BL/6 mice, whole body vibration was done at 45 Hz for 15min/day, seven days/week for five weeks with varying magnitudes.³⁸ A non-dose dependent response of bone volume was found when varying magnitudes.³⁸ In humans, whole body vibration was done at 30 Hz, which was found to be strongly anabolic in animal studies.³⁹ At this low frequency, prevention of bone loss was seen but no formation of bone was noted.³⁹

In addition to mechanical vibration, several other methods have been used to try to increase the rate of orthodontic tooth movement such as pulsed electromagnetic field driven vibration. Magnets are attached intra-orally into the specimen of interest, springs or coils are used to induce tooth movement, then an electromagnetic field is created to cause the vibration. Using this method with a frequency of 30 Hz in rats, orthodontic tooth movement was increased when coil springs were added.⁴⁰ In 1997, an electromagnetic field was used with a frequency of 15 Hz and orthodontic force of 15g to test orthodontic tooth movement in guinea pigs.⁴¹ At the end of the 10-day experimental period, the amount of tooth movement noted in the magnet and pulsed electromagnetic field groups was significantly greater than that of the group that used springs alone.⁴¹

The increase in the rate of tooth movement was suggested to be the result of a reduction of the initial lag phase when force is applied.⁴¹ However, it is impossible to distinguish the magnetic field's effect vs. the magnetic field driven force's effect. These results suggest that the presence of the magnetic field had induced multipotential stem cells to differentiate more rapidly into active osteoclasts, thereby increasing the rate of bone resorption and hence tooth movement.⁴¹

Stark and Sinclair used a stainless steel coil with a force of 12g and a frequency of 25 Hz.⁴² They found that at the end of 10 days, the experimental group had an additional 0.9 mm of tooth movement when compared to the controls.⁴⁰ Additionally, the incremental pattern of tooth movement found was similar to that seen in humans with an initial rapid movement, followed by a lag phase, and then a gradual increase for the remainder of the study.⁴²

The purpose of this study is two-fold: (1) to develop a standardized protocol for applying mechanical vibration to mice; (2) use this model to explore the hypothesis that mechanical vibration (4Hz frequency, 20micron displacement, 5min/session duration) increases OTM and decreases root resorption.

CHAPTER III: MATERIALS/METHODS

Subjects

This pilot study included thirty-six male, 20 week old, inbred strain C57BL/6 mice (Charles River Laboratories Inc., Wilmington, MA). Mice were acclimated for a minimum of 2 weeks to a 12-hour/light/dark cycle. Approval for the project was obtained through the IACUC at Marquette University, protocol # AR-218. No special eating or drinking patterns were imposed during this study. They were randomly assigned to 4 groups: control (n=6), spring only (n=10), vibration only (n=10), and spring and vibration (n=10). Mice were anesthetized using a combination of Ketamine (100mg/kg) / Xylazine (10mg/kg) placed as an intra-peritoneal injection. Anesthesia was given initially for spring placement and then repeated every third day for vibration application. Following the duration of the experiment (21 days), all mice were euthanized by CO₂ inhalation. The maxilla was dissected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) with springs in place for further examination.

Orthodontic Appliance

Springs (G & H Wire Company, Greenwood, IN), were fabricated using .010'' NiTi (Figure 4). Springs, in the shape of an omega loop, were placed across the palate with the terminal curve placed around the disto-lingual line angle on the maxillary 1st molars (Figures 5 and 6).

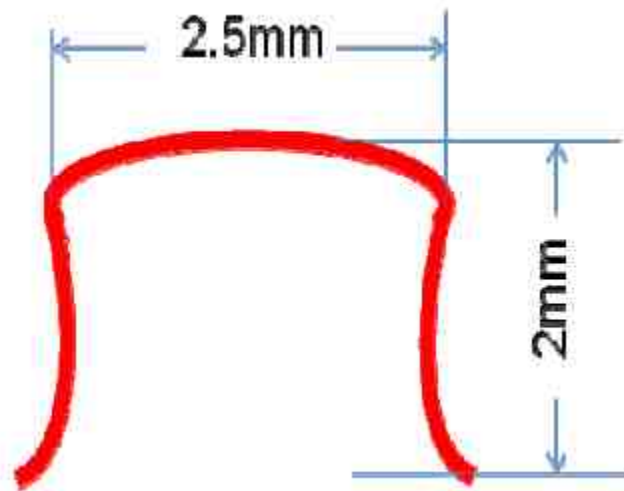


Figure 4: Diagram of approximate dimensions of NiTi spring.

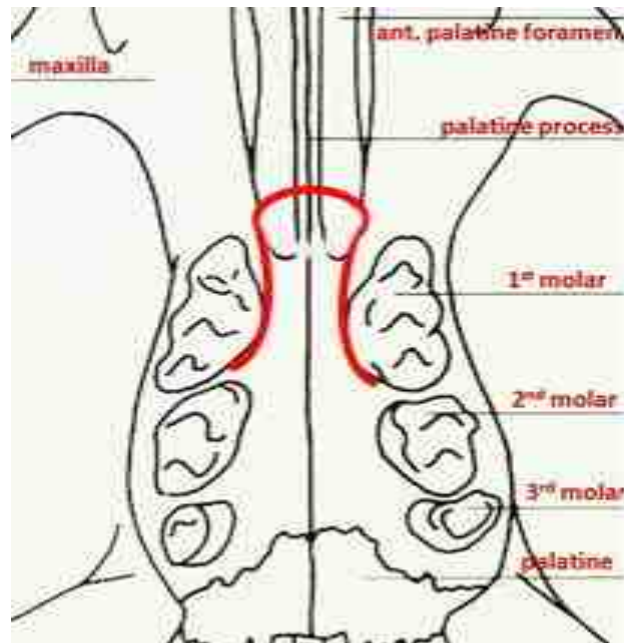


Figure 5: Schematic of NiTi spring placed across the palate between the maxillary 1st molars.



Figure 6: Photo of spring inserted in a mouse with a custom made mouth retractor holding the jaws apart.

The spring was secured by etching (self etching primer, 3M Unitek, Monrovia, CA) the enamel surface, followed by placement of light cure adhesive (flow-tain, Reliance, Itasca, IL). G & H wire constructed springs to deliver an initial 20 grams of force. The forces being delivered by the spring were tested by G & H with an MTS Insight 1, electro mechanical load cell and frame, manufactured by MTS (Eden Prairie, MN). Placement was aided by the use of a custom made retractor, .036'' stainless steel wire (GAC International Inc., Bohemia, NY).

Method of Vibration

In collaboration with the School of Engineering at Marquette University, a device was developed and fabricated to deliver vibration (Figure 7). Vibration was applied to animals in two groups, vibration only and spring/vibration, as a split mouth design (vibrated side vs. non-vibrated contra-lateral side). All vibration was performed on the maxillary left 1st molar with the maxillary right 1st molar serving as a control. The regimen for vibration was a frequency of 4 Hz, displacement of 20 μm , and 5 minutes per session. Sessions were repeated every three days, for a total of 7 sessions within the 12-day experimental period.

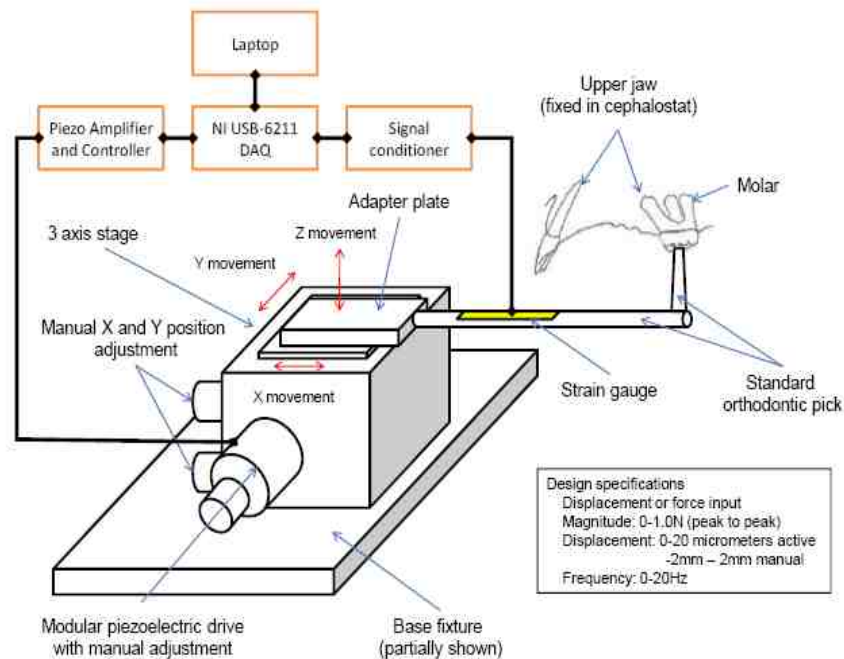


Figure 7: Diagram of vibration instrument.

μ CT Scans

The upper jaws/skull of the mice were scanned using a micro CT (μ CT) system at the Milwaukee VA hospital (Figure 8). The use of the μ CT system has been previously discussed and outlined by Karau et al⁴³, and will be reviewed in the following paragraphs.

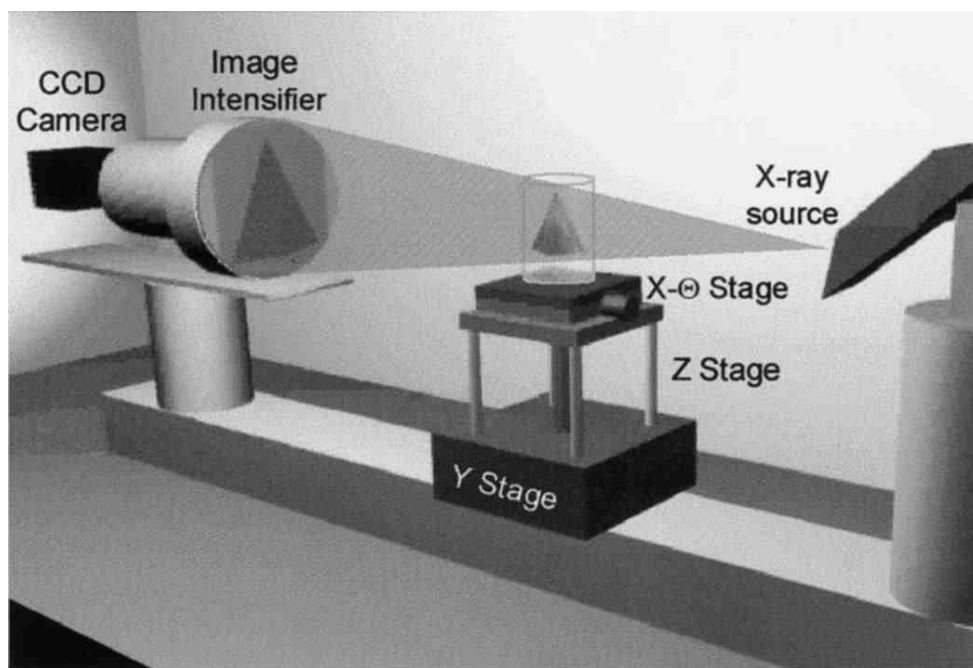


Figure 8

Figure 8: Diagram showing μ CT setup.⁴³

The μ CT x-ray system consisted of a micro-focal x-ray source in conjunction with an image-intensifier detector optically coupled to a digital charge-coupled device (CCD) camera. The x-ray was mounted on a precision optical rail, with the conical x-ray beam emerging in a horizontal orientation. The image intensifier was mounted on the rail with provision for movement along the beam axis with source-detector distance variable continuously between 30 and 150 cm. Also mounted on the rail, between the source and detector and adjustable between 0 and 120 cm, is a micro-positioning stage allowing animal preparations or other specimens to be manipulated in space with four degrees of

freedom, x-y-z- θ , providing the ability to acquire the desired x-ray projections through the specimen, variable magnification, and rotation for acquiring the image sequences for three dimensional reconstruction. Stage and camera operations were under the control of a Dell Precision 610 workstation. The physical design principle exploited to achieve high magnification, and thereby superior spatial resolution, is the combination of the very small x-ray source focal spot with the large-format, digital detector. In this imaging geometry, when the sample is placed in close proximity to the source and the detector some distance away, geometric magnification is achieved with minimal penumbral blurring. Available computing hardware include an Apple MacPro workstation, a Dell workstation, and several Pentium-based personal computers. These computers were connected to a local area network and have Internet access. These resources were accessible for signal and image processing, image reconstruction, and real-time data acquisition and analysis. Samples of mouse maxilla were fixed on the specimen stage and rotated in 1 degree increments to acquire 360 projection images. After each μ CT session, phantom images were taken to characterize and evaluate the aspects of the imaging acquisition and analysis. The phantom images included flood-field image with the mouse skull removed. The flood-field image was used to correct for spatial variations in the x-ray beam and/or image-intensifier gain. The second image was of a uniform grid of 1-mm diameter stainless steel spheres (BB's) spaced at 1.5-cm intervals and embedded in a Plexiglas disk (Figure 9). The BB phantom image was used to correct for spatial distortion due to the beam geometry and image intensifier.

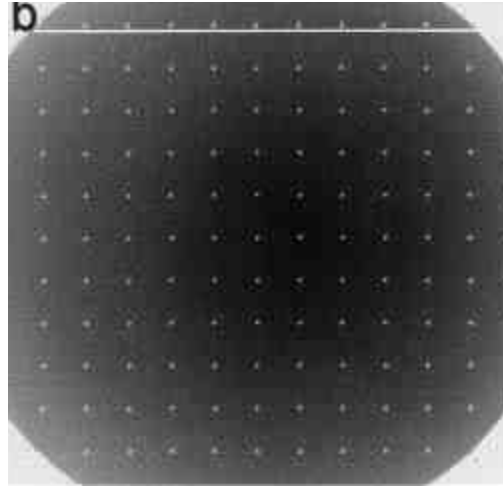


Figure 9: Diagram of BB phantom.⁴³

Reconstruction

High spatial resolution 3-D volume data was reconstructed using the FeldKamp cone-beam algorithm. As explained by Karau et al, prior to reconstructing the image volume from the 360 planar images, preprocessing of the individual images was performed in the following steps: 1) two-dimensional polynomial dewarping to correct the image-intensifier spatial distortion, 2) locating the axis of rotation and cropping the projection images to center on that axis, 3) flood-field division to correct for nonuniform illumination intensity, and 4) normalization of the intensity between projections to correct for any temporal drift.⁴³ Preprocessing produced a final image size of 497x497 pixels.⁴³

Reconstruction was done using X-11(X_windows) and Image J (downloaded from <http://rsbweb.nih.gov/ij/>). Using these programs reconstruction was performed on the projection data to yield an isotropic reconstruction matrix of 497x497x497 voxels.⁴³

Measurements

Micro-CT scans were reconstructed. Axial and coronal views were used to measure the various structures using Analyze (Biomedical Imaging Resource, Mayo Clinic, MN).^{44,45,46} Five random specimens were selected to determine intra-rater reliability. The Five specimens were measured by the same examiner at three different time points with a minimum of 1 week in between.

Coronal View

The reconstruction was manipulated to provide a view of the palatal root on the maxillary 1st molar. The image was adjusted by using in/out, up/down, left/right, and rotation adjustments to provide an image where the left portion of the image was nearly identical to the right portion of the image (dividing line for the left/right was done by the mid line of the skull). The image was considered suitable if the crown anatomy, root anatomy, skull symmetry, and open apex were similar for both the right and left sides. Left and right sides were determined by scrolling through slices to determine the sequence of anatomy, such as where the 2nd and 3rd molars were located, or by the direction of the spring if present. Once the image was obtained, the coordinates were saved allowing that particular image to be brought up for further measuring.

Coronal Measurements

Assuming the midline of the skull did not change during the experiment, several measurements used the midline to provide a reliable reference in order to compare measurements and gather information. Midline structures may include vomer, perpendicular plate of ethmoid, and palatal suture (Figure 10).

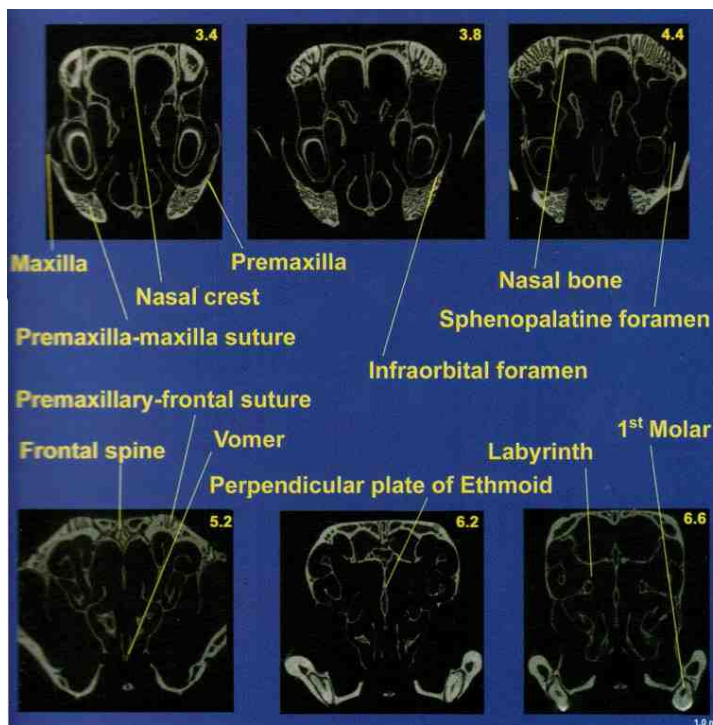


Figure 10: Diagram demonstrates midline structures used for measuring.²

1. **Midskull / CEJ:** Distance from lingual CEJ to midline of the skull.

Measurement is taken from CEJ along line to contact point with midline line. Left and right sides measured (Figure 11).

2. **Midskull / Long axis of tooth:** Angle formed between the long axis of the palatal root of the maxillary 1st molar and midline of the skull. Left and right sides measured, provides the amount of tipping (Figure 11).
3. **Midskull / occlusal table:** Angle formed between a line representing the occlusal table and the midline of the skull. Left and right sides were measured, provides the amount of tipping (Figure 11).
4. **Root Length at 90:** Base line formed from connecting lingual and buccal CEJ's. A second line is drawn at a 90 degree angle to the base line down to the most apical portion of the root. Measurement is taken from apical portion of root to where long axis line bisects CEJ line. Left and right sides were measured, provides length of (Figure 11).
5. **PDL width at 90:** Base line is connecting lingual and buccal CEJ's. Two lines are then drawn to form a 90 degree angle with the line connecting the CEJ's; one to most apical portion of root, second is to the alveolar bone. Measurement is taken from the end point of each long axis line to where the line bisects with CEJ line. The difference between the two lines is recorded. Left and right sides were measured, provides width of periodontal ligament (Figure 11).



Figure 11: Diagram representing the various coronal measurements.

Axial View

The reconstruction was manipulated to provide a cross section of the left and right 1st, 2nd, and 3rd maxillary molar roots. The image was adjusted by using in/out, up/down, left/right, and rotation adjustments to provide a reliable view of the palate. When reviewing the anatomy of the mouse, it was determined that when the palate was in full view it would provide as a reliable marker that the image was showing a cross section of the 1st molar roots halfway down the root structure.² The image was considered suitable if the root anatomy, skull symmetry, and clear view of the palate were similar for both the right and left

sides. Left and right sides were determined by scrolling through slices to determine the sequence of anatomy, such as where which direction the apex or crown was, or by the direction of the spring if present. Once the image was obtained, the coordinates were saved allowing that particular image to be brought up for further measuring.

Axial measurements

Assuming the midline of the skull did not change during the experiment, several measurements used the midline to provide a reliable marker in order to compare measurements and gather information (Figure 12).

1. **Palatal suture width:** The width of the palatal suture was measured (mm). The suture for each specimen was measured adjacent to the palatal root of the maxillary 1st molar in order to have a standardized point for measuring (Figure 12).
2. **Midskull / buccal root:** Angle formed by a line through the midline of the skull and a line bisecting the center of disto-buccal and mesio-buccal roots. Left and right sides were measured, used to provide information on tipping movement (Figure 12).
3. **PDL width buccal:** Two lines were drawn bisecting the center of mesio-buccal root and forming a 90 degree angle with midline. The first line was drawn to the most buccal portion of the PDL space, while the second line was drawn to the most buccal

portion of the root. Distance between the two lines was the width of the buccal PDL space (mm). Left and right sides measured (Figure 12).

4. **Midskull to buccal plate:** A line was then drawn through the center of the mesio-buccal root of the 1st molar to the most buccal portion of the buccal plate, perpendicular to midline. The distance (mm) from the buccal plate to midline was recorded. Left and right sides were measured (Figure 12).

5. **PDL width lingual:** Two lines were then drawn bisecting the center of mesio-buccal root and forming a 90 degree angle with the midline. The first line was drawn to the most lingual portion of the root, while the second line was drawn to the most lingual portion of the PDL. The difference between the two lines gave the width (mm) of the lingual PDL. Left and right sides measured. (not shown)

6. **Midskull to lingual plate:** A line was then drawn through the center of the mesio-buccal root of the 1st molar, perpendicular to the midline. The distance (mm) from the most lingual portion of the PDL to the midline was recorded. Left and right sides were measured (not shown).

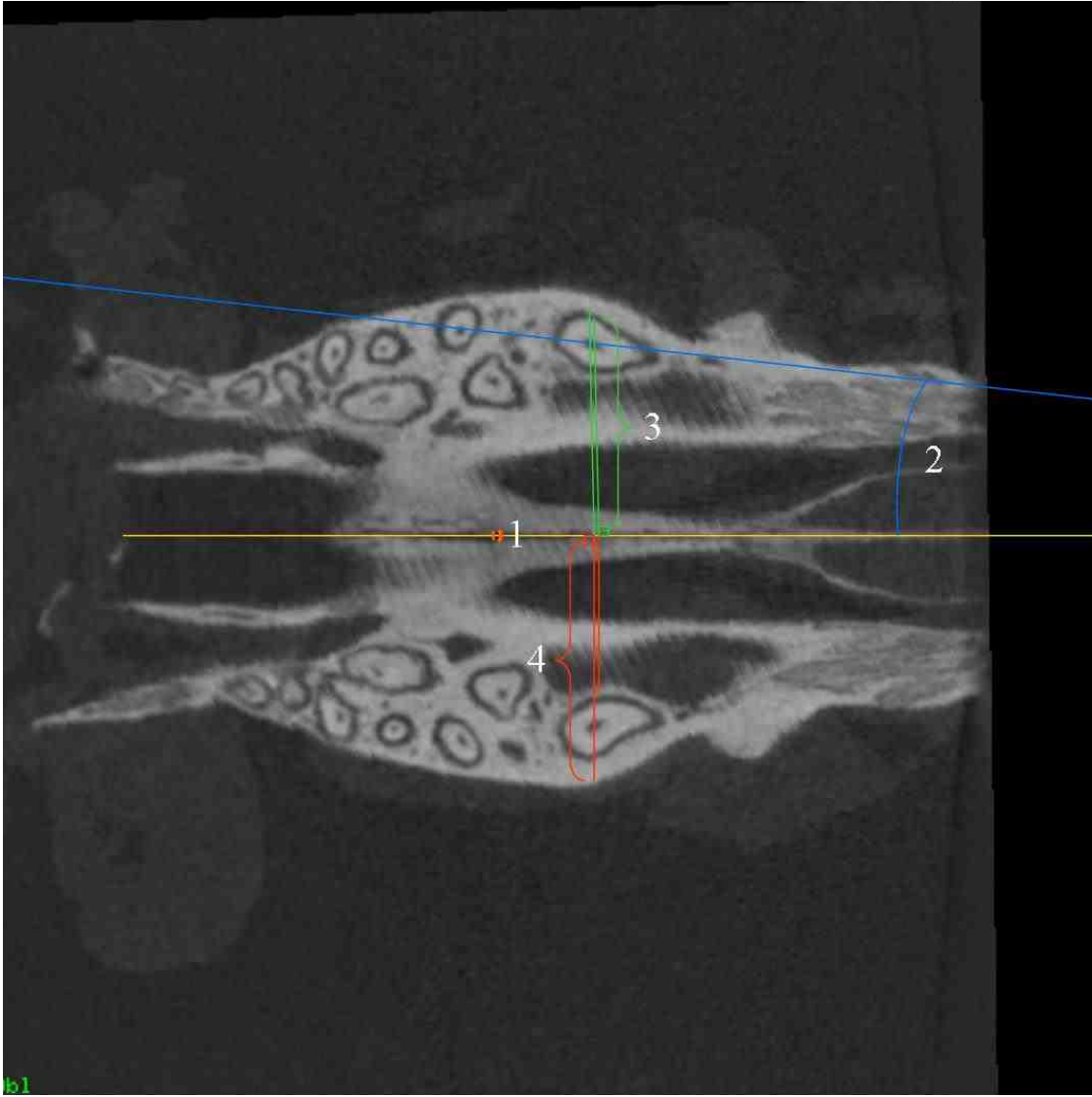


Figure 12: Diagram representing the various axial measurements.

Statistical Analysis

Means and standard deviations were calculated for all measured variables and for the differences between paired variables (left – right). One-way analysis of variance (ANOVA) was used to test the significance of differences among treatment groups. Dunnett's test was used to assess comparisons between groups when the omnibus test

(ANOVA) was significant ($P < 0.05$). This test is useful when group variances show substantial differences. Differences between left side and right side within a treatment group were analyzed using the one-sample t-test. Intra-examiner reliability was assessed using the intra-class correlation coefficients as described by Shrout and Fleiss (1979).⁴⁷ All computations were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL)

CHAPTER IV: RESULTS

Intra examiner reliability

Intra examiner reliability was completed for all variables (left and right sides). Outcomes were considered highly reliable when intra-class correlation coefficient (ICC)>0.8 and moderately reliable when ICC ranged from 0.5 to 0.8. Variables with an intra-class correlation coefficient of less than .499 were considered unreliable. Those variables were: root resorption by long axis (mm) (left and right) and width of mid-palatal suture (mm).

Results of variable measurements

Results for the variables measured are shown below in tables 1-11 and figures 13-33.

Variables on coronal plane of space**1. Midskull / CEJ (mm)**

The control and vibration groups were significantly different than the spring and the spring+vibration groups ($p<0.05$).

Table 1. Results of Midskull / CEJ (distance in mm)

	Side	Mean	Std. Deviation	N
Control	Right	1.475	.0437	6
	Left	1.491	.0499	6
	<i>Diff</i>	<i>0.0177</i>	<i>0.0377</i>	6
Spring	Right	1.746	.133	10
	Left	1.856	.180	10
	<i>Diff</i>	<i>0.110</i>	<i>0.282</i>	10
Spring+Vibration	Right	1.750	.0910	10
	Left	1.897	.214	10
	<i>Diff</i>	<i>0.147</i>	<i>0.282</i>	10
Vibration	Right	1.422	.0405	10
	Left	1.457	.0368	10
	<i>Diff</i>	<i>0.035</i>	<i>0.070</i>	10

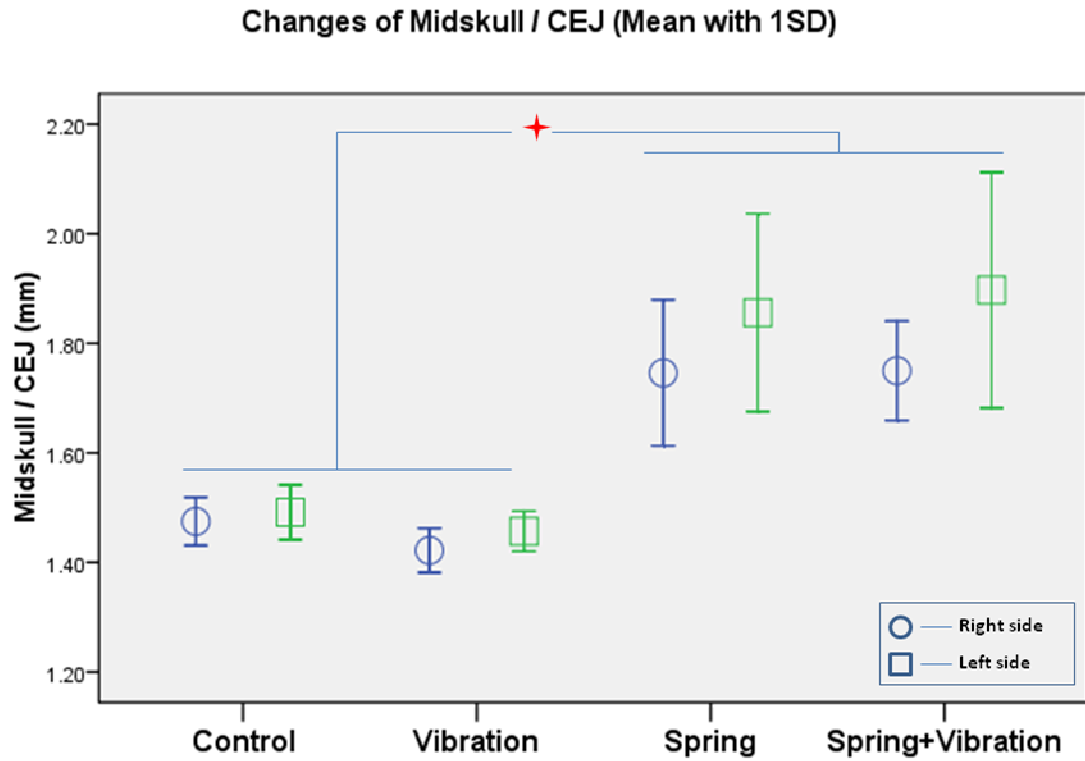


Figure 13: Graph depicting Mid-skull / CEJ length for left and right sides for each group. Data were presented as mean \pm 1SD. (p-value <0.05).

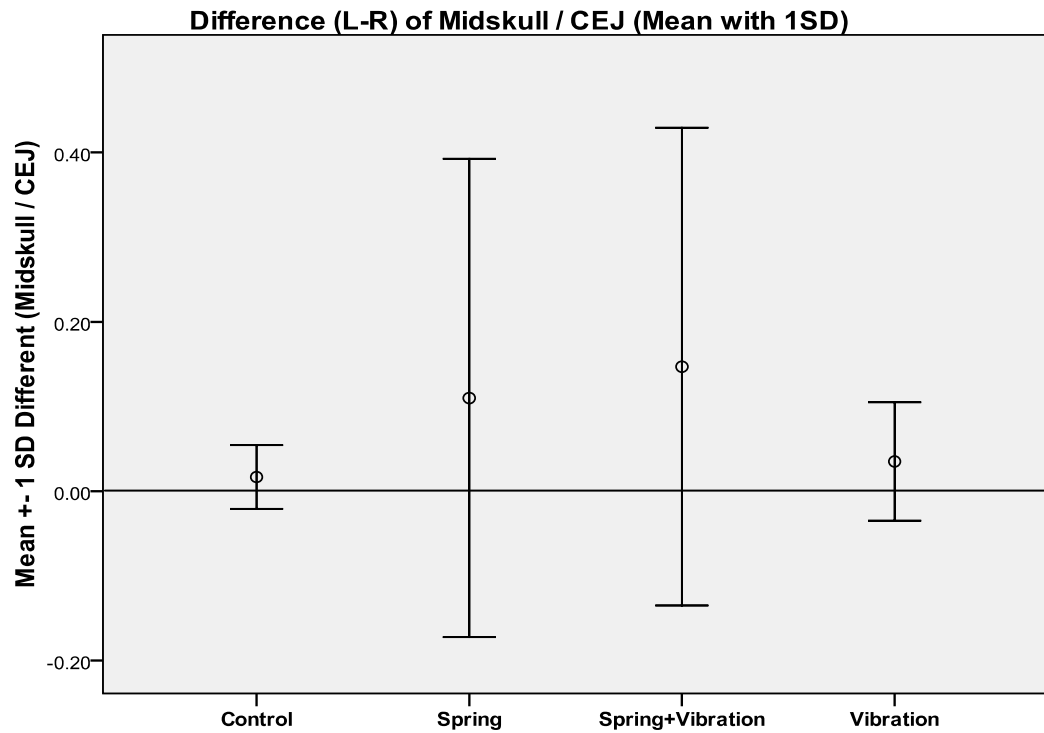


Figure 14: Graph depicting Mid-skull / CEJ length differences between left side and right side (L-R) for each group. Data were presented as $\text{mean} \pm 1\text{SD}$.

2. Midskull / Long axis of tooth (degree)

Mean values for groups “Control” and “Vibration” were similar. A treatment effect was observed for groups “Spring” and “Spring+Vibration”. Any difference observed between “left” and “right” sides was not statistically significant.

Table 2: Results of Midskull / Long axis of tooth (angle in degree)

	Side	Mean	Std. Deviation	N
Control	Right	12.525	1.534	6
	Left	11.665	2.682	6
	<i>Diff</i>	<i>-0.860</i>	<i>3.957</i>	6
Spring	Right	27.543	4.815	10
	Left	25.449	8.515	10
	<i>Diff</i>	<i>-2.094</i>	<i>10.763</i>	10
Spring+Vibration	Right	26.679	5.453	10
	Left	27.974	8.146	10
	<i>Diff</i>	<i>1.295</i>	<i>11.621</i>	10
Vibration	Right	12.071	2.645	10
	Left	12.292	2.795	10
	<i>Diff</i>	<i>0.221</i>	<i>3.478</i>	10

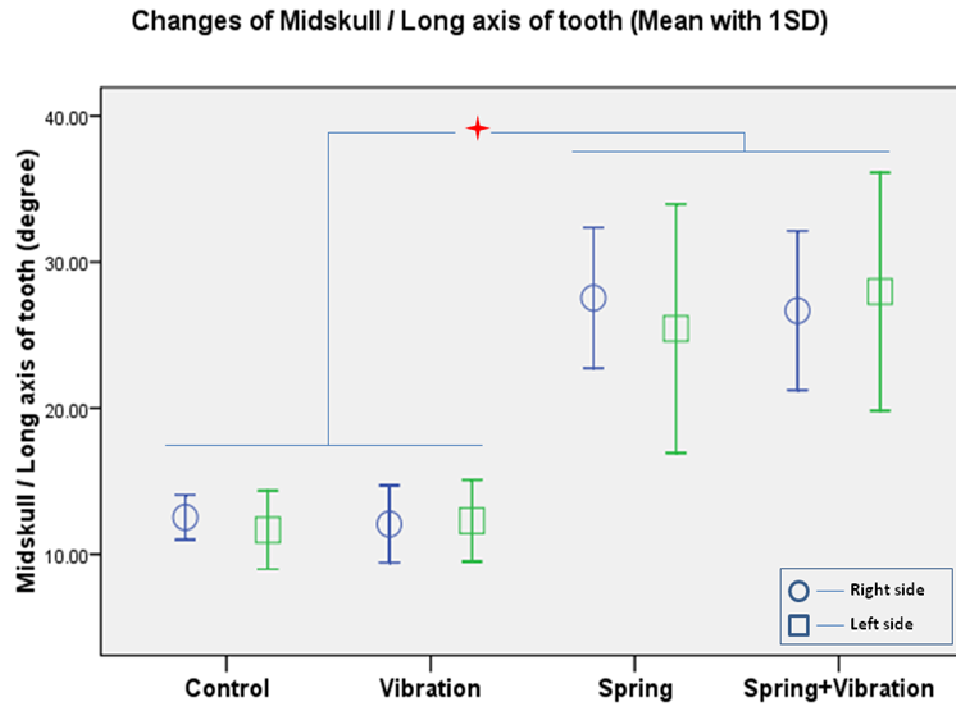


Figure 15: Graph depicting Mid-skull / CEJ length for left and right sides for each group. Data were presented as mean \pm 1SD. (p-value <0.05).

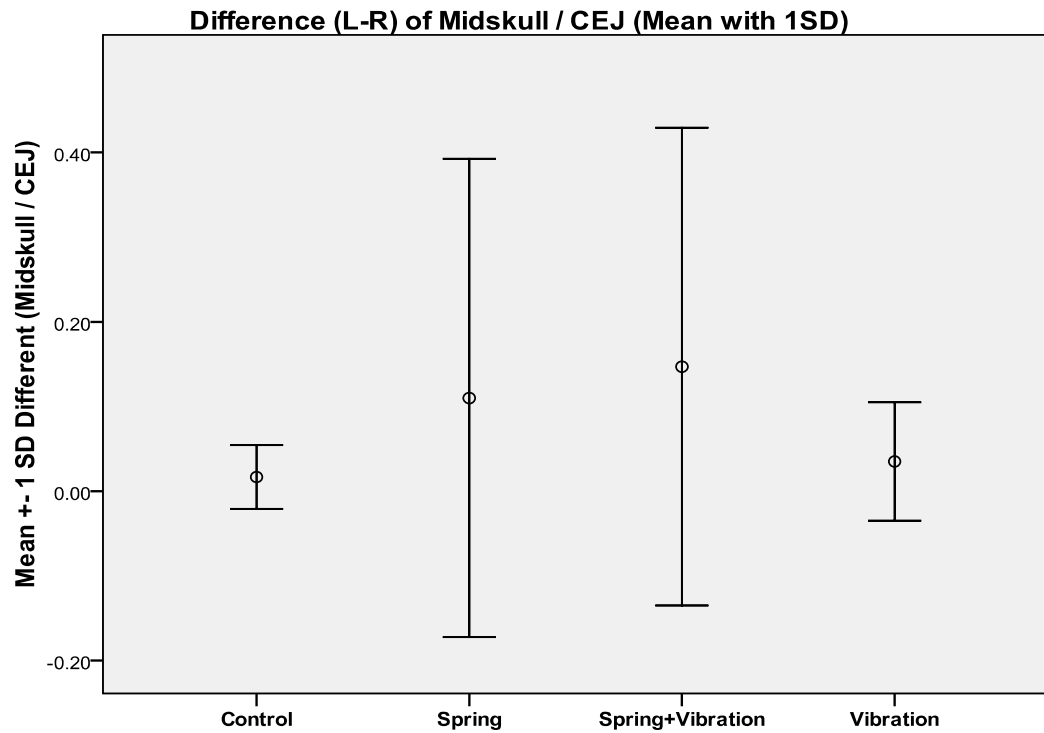


Figure 16: Graph depicting Mid-skull / CEJ length differences between left side and right side (L-R) for each group. Data were presented as $\text{mean} \pm 1\text{SD}$.

3. Midskull / Occlusal table (degree)

Mean angle size was comparable for “Control” and “Vibration”. As expected, Spring treatment resulted in a smaller angle as seen in “Spring” and “Spring+Vibration” ($p < 0.05$). Within “Spring+Vibration” group, the effect of vibration treatment was indicated by a trend towards further angle reduction.

Table 3. Results of Midskull / Occlusal table (angle in degree)

	Side	Mean	Std. Deviation	N
Control	Right	84.561	4.251	6
	Left	84.176	1.906	6
	<i>Diff</i>	<i>-0.385</i>	<i>5.667</i>	6
Spring	Right	64.475	5.353	10
	Left	65.464	6.978	10
	<i>Diff</i>	<i>0.989</i>	<i>8.949</i>	10
Spring+Vibration	Right	68.129	3.267	10
	Left	63.778	10.123	10
	<i>Diff</i>	<i>-4.351</i>	<i>9.8346</i>	10
Vibration	Right	85.359	2.166	10
	Left	84.524	2.613	10
	<i>Diff</i>	<i>-0.835</i>	<i>4.011</i>	10

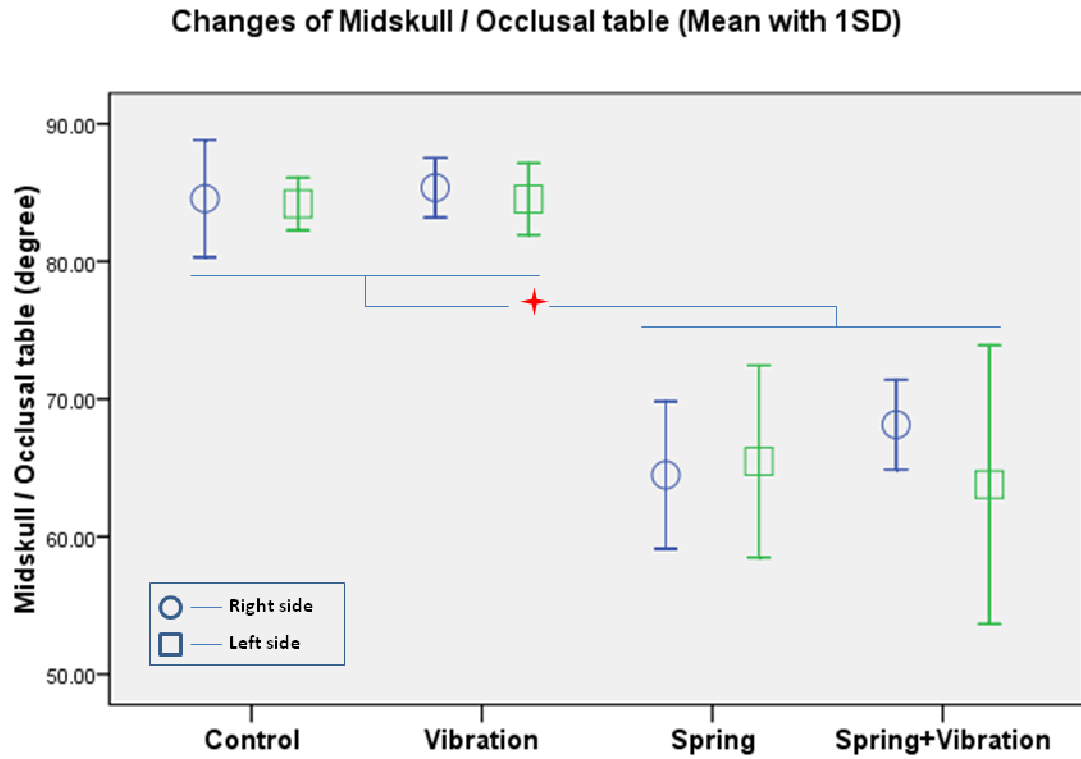


Figure 17. Graph depicting Midskull / occlusal table for left and right sides for each group. Data were presented as mean \pm 1SD. (p-value <0.05).

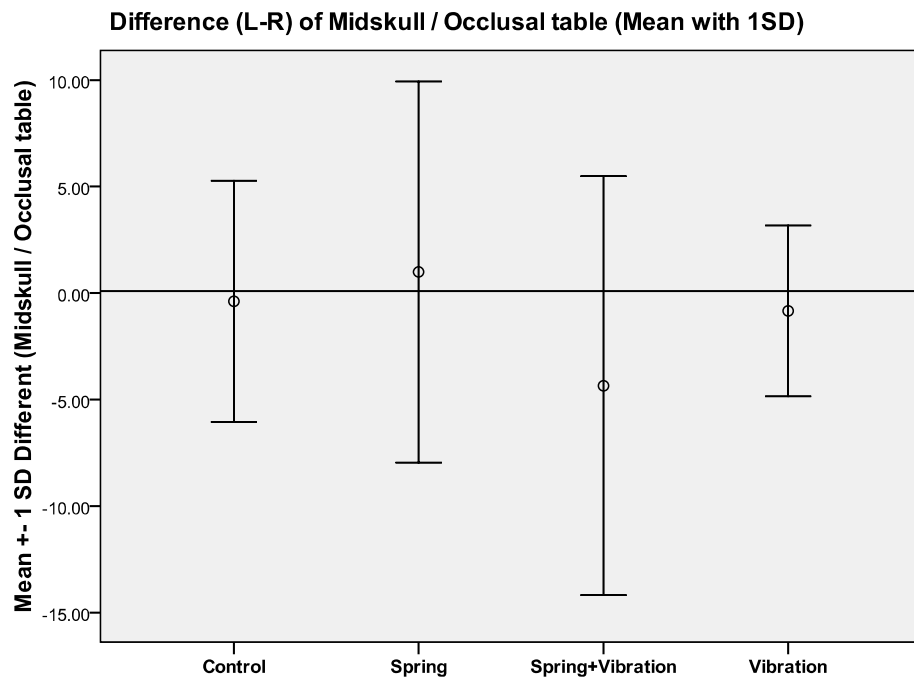


Figure 18. Graph depicting mid-skull / occlusal table differences between left side and right side (L-R) for each group. Data were presented as $\text{mean} \pm 1\text{SD}$.

4. Root Length at 90 (distance in mm)

There was a statistically significant ($p < 0.05$) difference for this outcome among treatment groups.

Table 4. Results of Root Length at 90 (distance in mm)

	Side	Mean	Std. Deviation	N
Control	Right	1.136	.0136	6
	Left	1.103	.00816	6
	<i>Diff</i>	<i>-0.03</i>	<i>0.020</i>	6
Spring	Right	1.108	.0446	10
	Left	1.144	.0587	10
	<i>Diff</i>	<i>0.044</i>	<i>0.074</i>	10
Spring+Vibration	Right	1.123	.0505	10
	Left	1.158	.0373	10
	<i>Diff</i>	<i>0.025</i>	<i>0.040</i>	10
Vibration	Right	1.084	.0347	10
	Left	1.081	.0360	10
	<i>Diff</i>	<i>0.003</i>	<i>0.048</i>	10

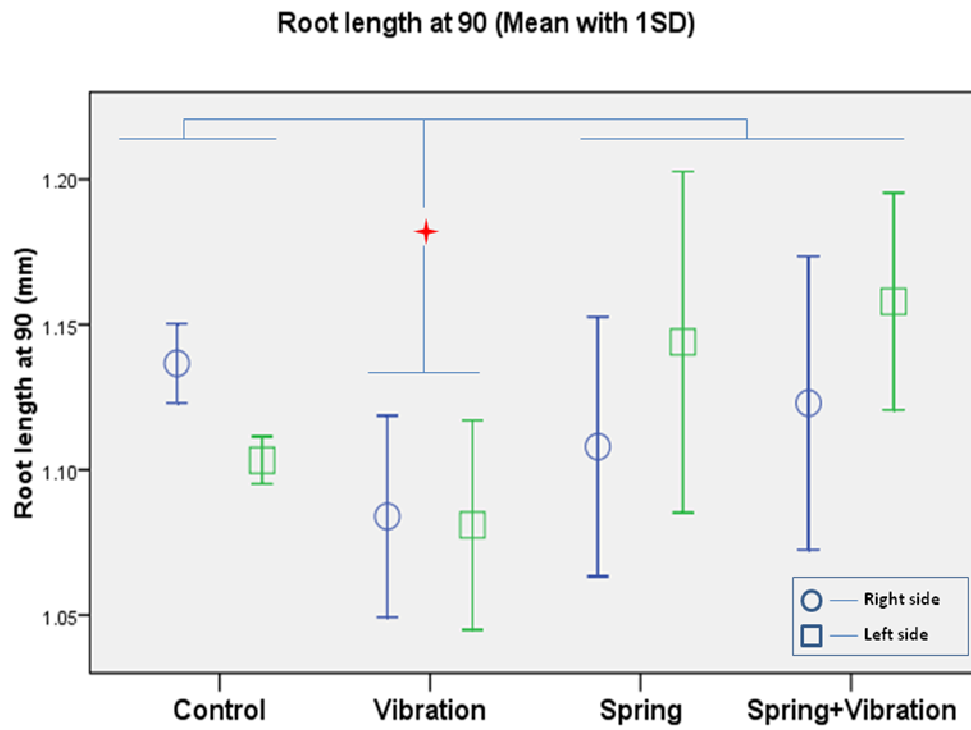


Figure 19. Graph depicting RR at 90 for left and right sides for each group. Data were presented as mean \pm 1SD. (p-value <0.05).

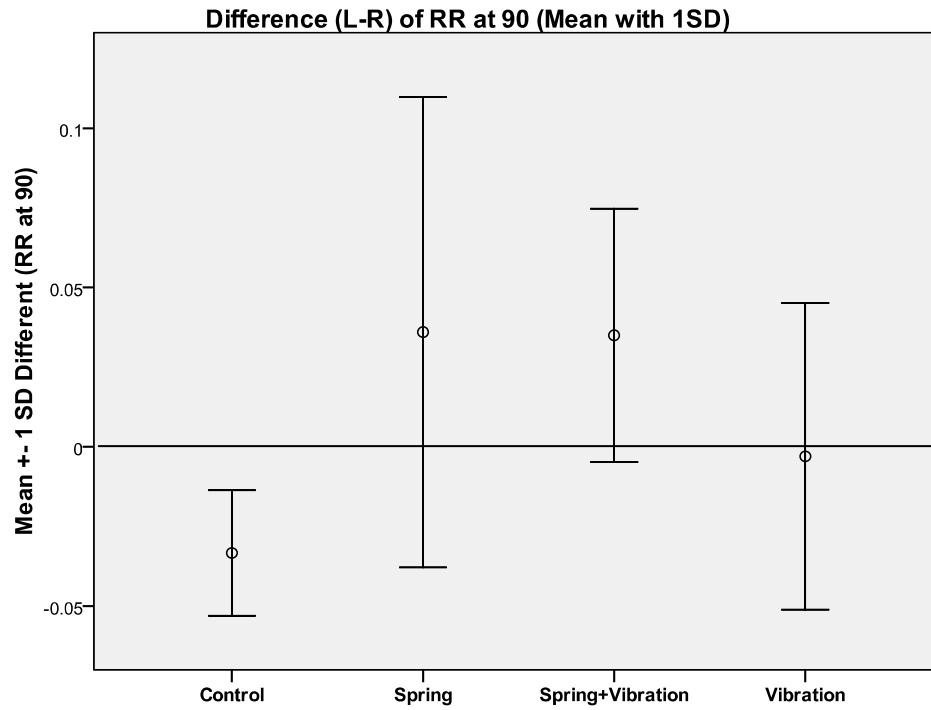


Figure 20. Graph depicting RR at 90 differences between left side and right side (L-R) for each group. Data were presented as mean \pm 1SD.

5. PDL width at 90 (distance in mm)

There was a statistically significant ($p < 0.01$) tendency towards greater PDL width in treatment groups “Spring” and “Spring+Vibration” from “Control” and “Vibration”. No difference was observed between left and right in any treatment group.

Table 5: Results of PDL width (**distance in mm**)

	Side	Mean	Std. Deviation	N
Control	Right	.0683	.0204	6
	Left	.0850	.0207	6
	<i>Diff</i>	<i>0.027</i>	<i>0.035</i>	6
Spring	Right	.157	.0290	10
	Left	.145	.0359	10
	<i>Diff</i>	<i>-0.015</i>	<i>0.0474</i>	10
Spring+Vibration	Right	.151	.0360	10
	Left	.155	.0432	10
	<i>Diff</i>	<i>0.015</i>	<i>0.0619</i>	10
Vibration	Right	.0880	.0154	10
	Left	.0960	.0217	10
	<i>Diff</i>	<i>0.007</i>	<i>0.0271</i>	10

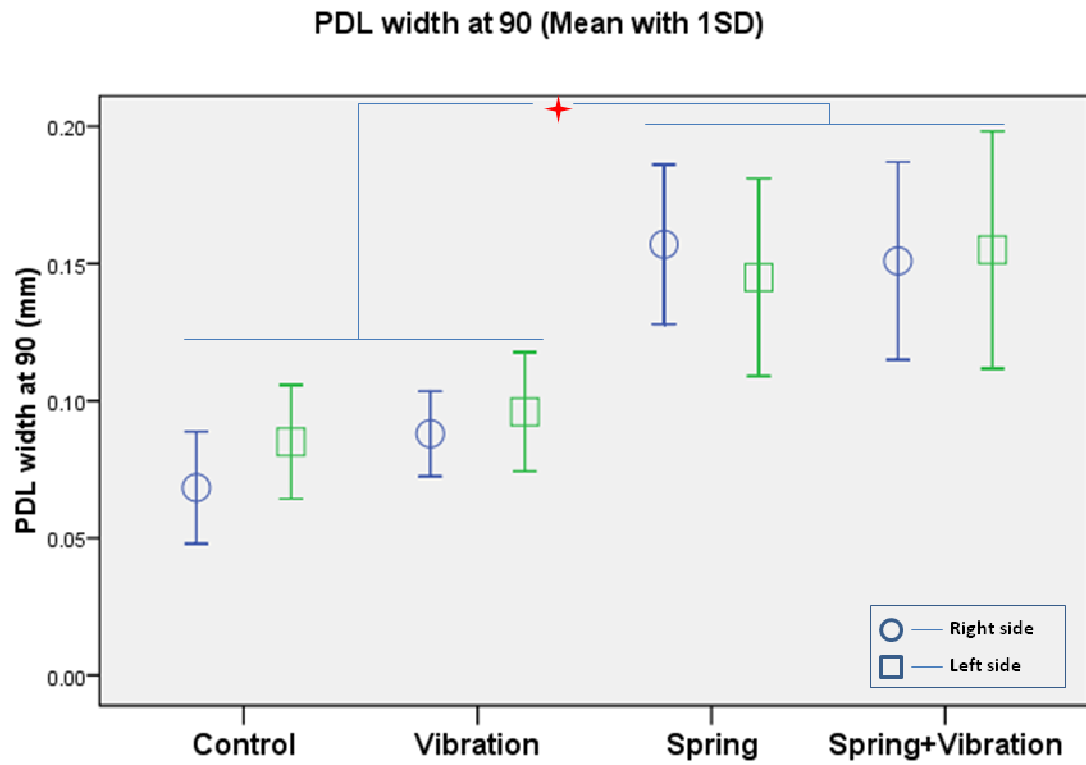


Figure 21. Graph depicting PDL width at 90 for left and right sides for each group. Data were presented as mean \pm 1SD. (p-value <0.05).

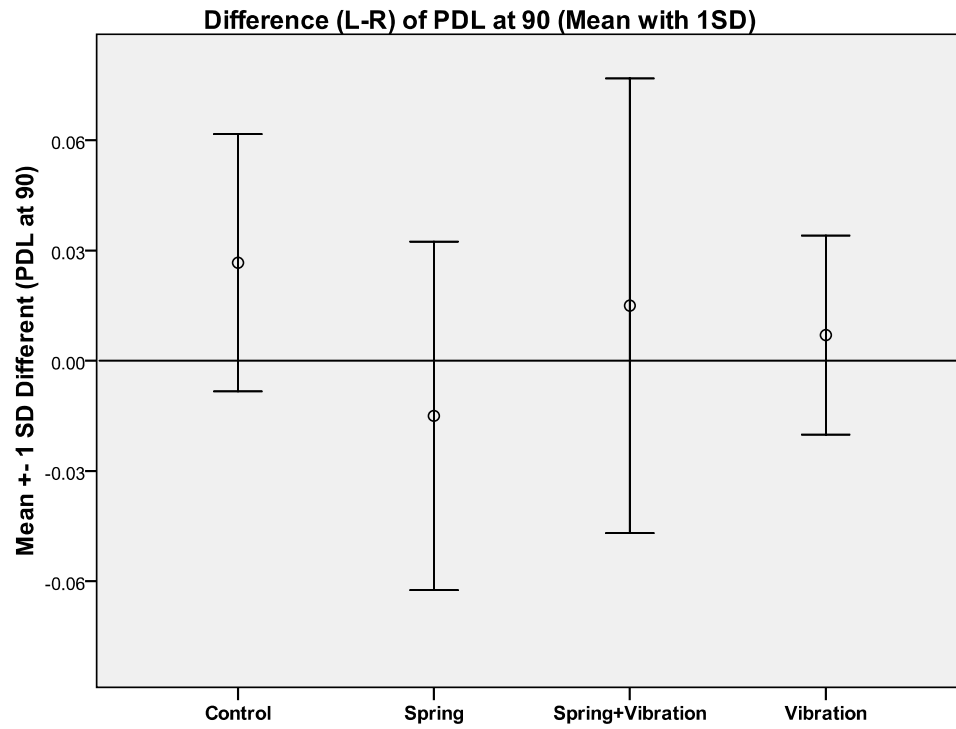


Figure 22. Graph depicting PDL at 90 differences between left side and right side (L-R) for each group. Data were presented as mean \pm 1SD.

Variables on axial plane of space

1. Palatal suture width (distance in mm)

A statistically significant ($p < 0.05$) treatment effect was observed in groups “Spring” and “Spring+Vibration”. The difference between “Control” and “Vibration” was explained by chance ($p > 0.5$). Similarly, the difference between “Spring” and “Spring+Vibration” was not statistically significant.

Table 6: Results for Palatal suture width (**distance in mm**)

Group	Mean	Std. Deviation	N
Control	.0683	.0160	6
Spring	.185	.0432	10
Spring+Vibration	.188	.0949	10
Vibration	.0940	.0157	10

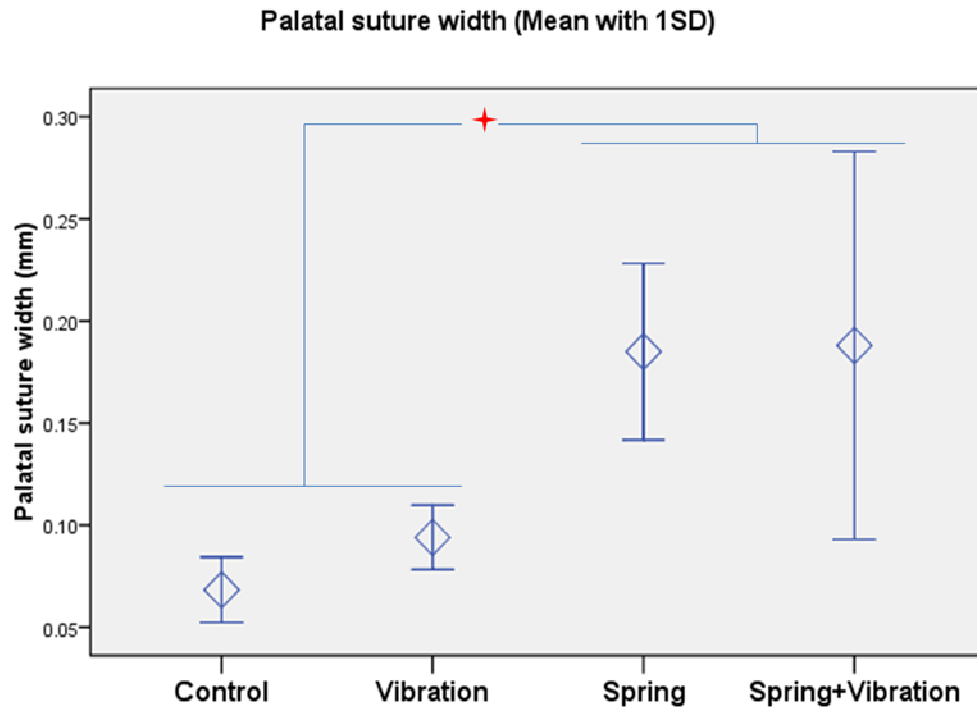


Figure 23: Graph depicting mid-palatal suture width for each group. Data were presented as mean \pm 1SD. (p-value <0.05).

2. Midskull / buccal root (degree)

A statistically significant ($p < 0.05$) treatment effect was observed between the groups that had spring activation and those that did not. The difference between “Spring” and “Spring+Vibration” was not statistically significant.

Table 7: Results of Midskull / buccal root

	Side	Mean	Std. Deviation	N
Control	Right	4.775	.923	6
	Left	4.905	.597	6
	<i>Diff</i>	0.130	0.7399	6
Spring	Right	9.089	3.598	10
	Left	10.118	4.630	10
	<i>Diff</i>	1.029	2.591	10
Spring+Vibration	Right	9.097	3.390	10
	Left	10.030	4.388	10
	<i>Diff</i>	0.933	2.067	10
Vibration	Right	4.536	1.169	10
	Left	4.400	1.423	10
	<i>Diff</i>	-0.136	1.7165	10

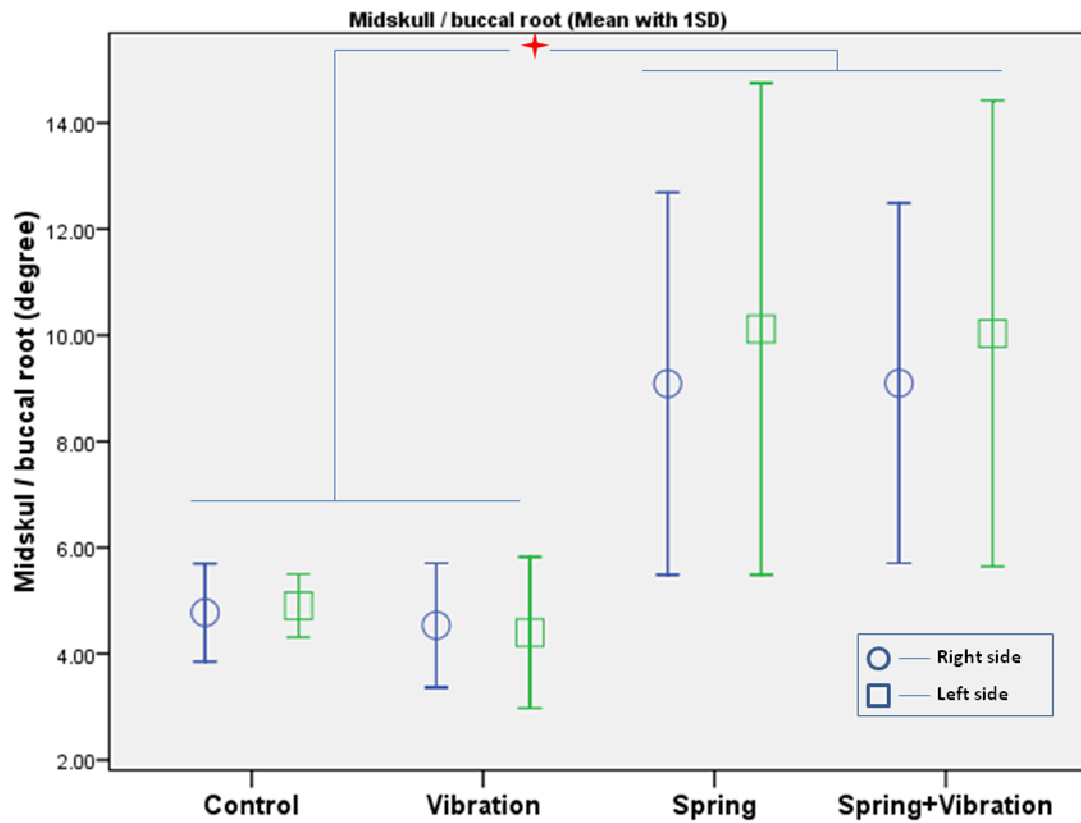


Figure 24: Graph depicting angle formed by Midskull / buccal roots of 1st molar for left and right sides in each group. Data were presented as mean \pm 1SD.

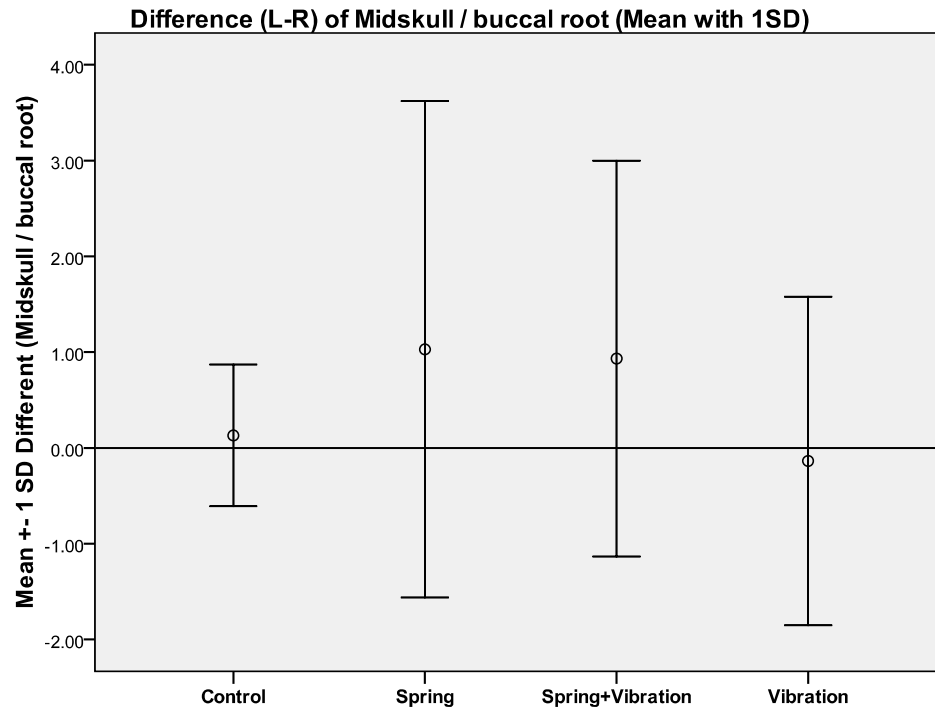


Figure 25. Graph depicting mid-skull / buccal root differences between left side and right side (L-R) for each group. Data were presented as $\text{mean} \pm 1\text{SD}$.

3. PDL width buccal (mm)

A statistically significant ($p < 0.05$) treatment effect was observed only in the Control group. The difference between “Spring” and “Spring+Vibration” was not statistically significant.

Table 8: Results of PDL width buccal

	Side	Mean	Std. Deviation	N
Control	Right	.0717	.0160	6
	Left	.0667	.0225	6
	<i>Diff</i>	-0.005	0.0321	6
Spring	Right	.0980	.0301	10
	Left	.0989	.0284	10
	<i>Diff</i>	0.002	0.0323	10
Spring+Vibration	Right	.103	.0374	10
	Left	.109	.0395	10
	<i>Diff</i>	0.006	0.0384	10
Vibration	Right	.0930	.0188	10
	Left	.0920	.0122	10
	<i>Diff</i>	-0.001	0.02685	10

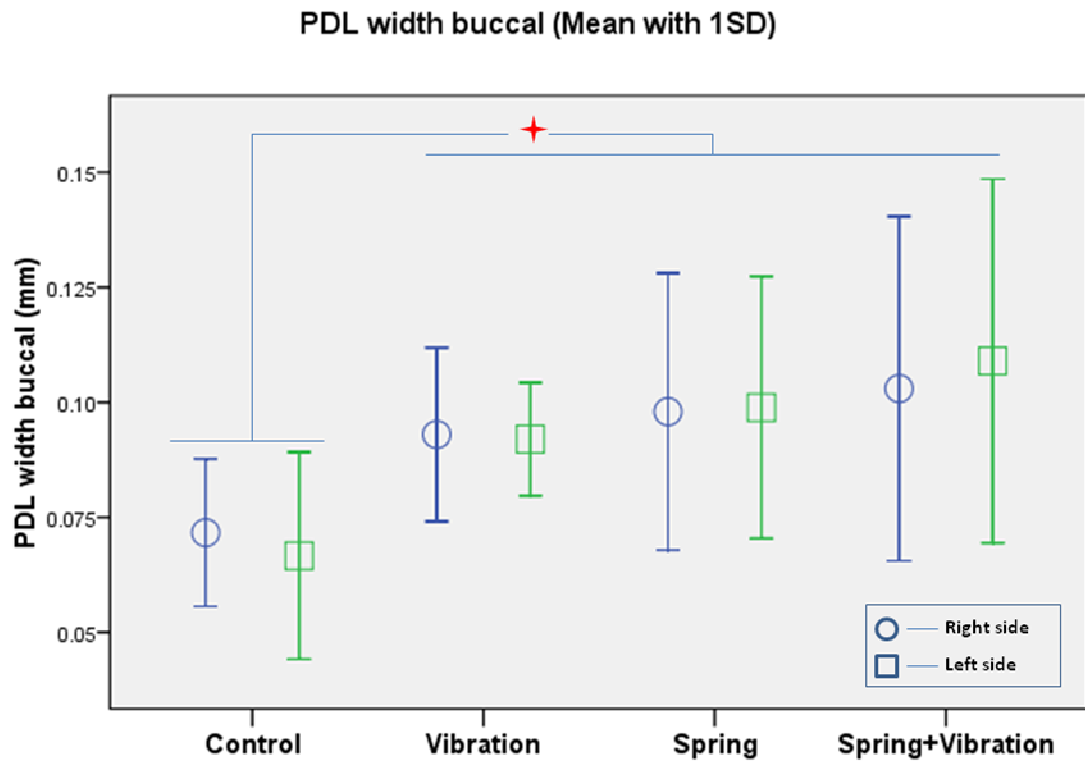


Figure 26: Graph depicting width of buccal PDL of mesiobuccal root on 1st molar for left and right sides in each group. Data were presented as mean±1SD. (p-value <0.05).

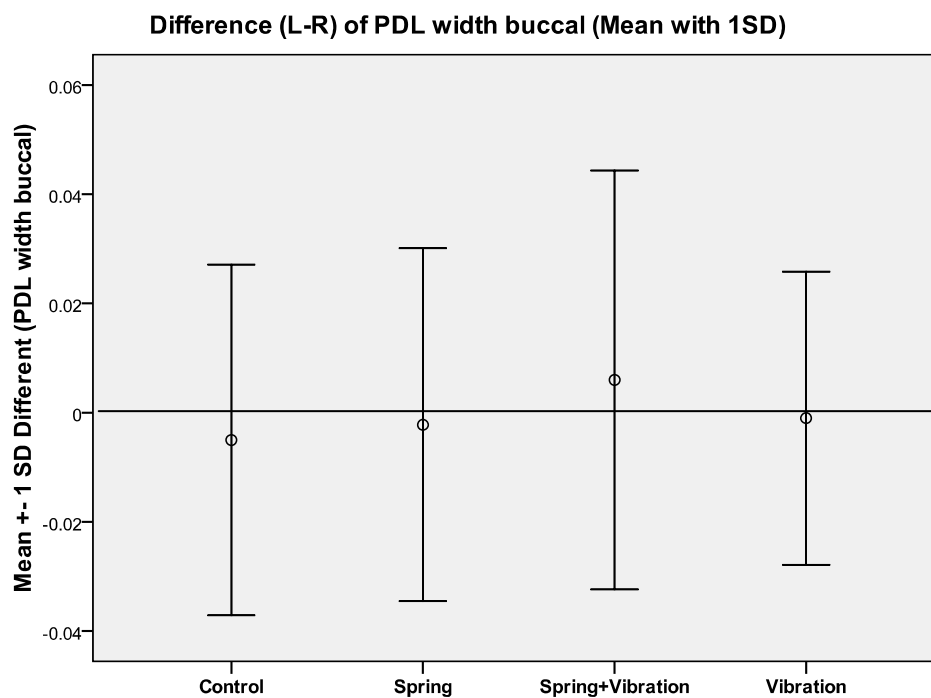


Figure 27. Graph depicting PDL width buccal differences between left side and right side (L-R) for each group. Data were presented as $\text{mean} \pm 1\text{SD}$.

4. Midskull to buccal plate (mm)

A statistically significant ($p < 0.05$) treatment effect was observed between the vibration group and the other three groups.

Table 9: Results of Midskull to buccal plate

	Side	Mean	Std. Deviation	N
Control	Right	2.585	.134	6
	Left	2.566	.0484	6
	<i>Diff</i>	-0.1833	0.1175	6
Spring	Right	2.517	.0377	10
	Left	2.522	.0438	10
	<i>Diff</i>	0.0111	0.03655	10
Spring+Vibration	Right	2.533	.0666	10
	Left	2.539	.0570	10
	<i>Diff</i>	0.006	0.0401	10
Vibration	Right	2.461	.0409	10
	Left	2.470	.0496	10
	<i>Diff</i>	0.009	0.0484	10

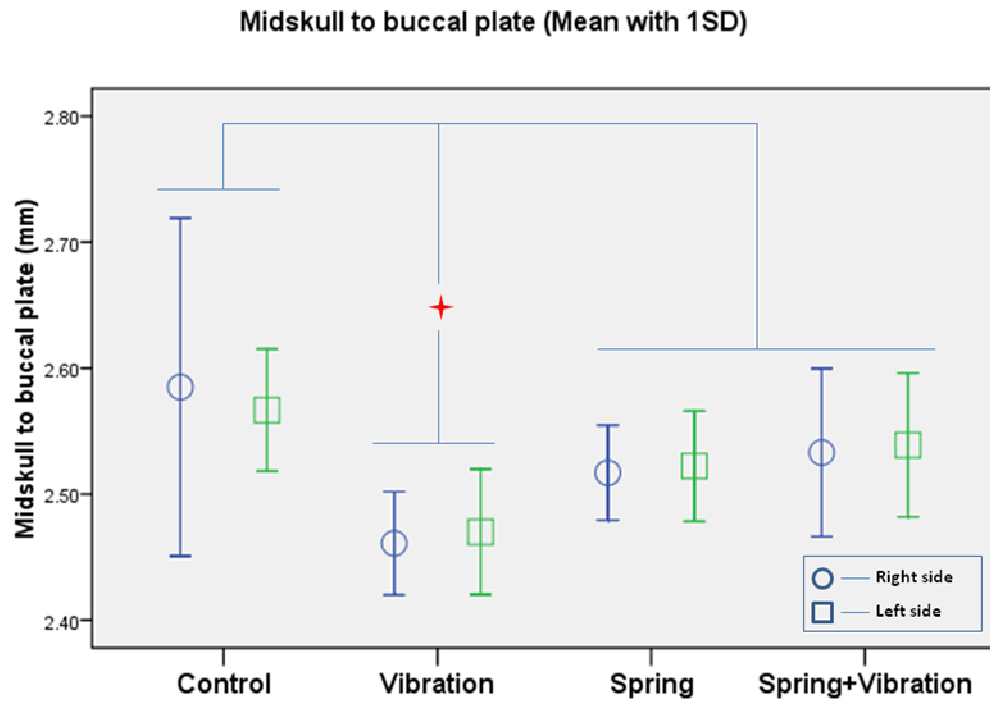


Figure 28: Graph depicting length from midskull to buccal portion of buccal plate at 1st molar mesio-buccal root for left and right sides in each group. Data were presented as mean±1SD. (p-value <0.05).

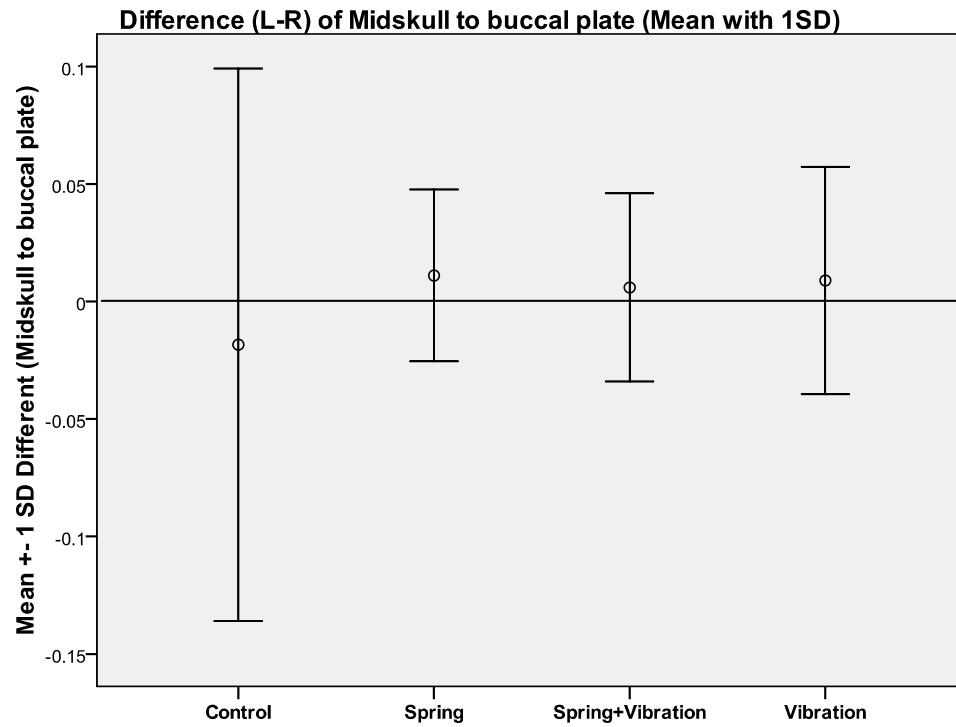


Figure 29. Graph depicting Mid-skull / buccal plate differences between left side and right side (L-R) for each group. Data were presented as mean \pm 1SD.

5. PDL width lingual (mm)

A statistically significant ($p < 0.05$) treatment effect was observed between the control group and the other three groups.

Table 10: Results of PDL width lingual

	Side	Mean	Std. Deviation	N
Control	Right	.0617	.0160	6
	Left	.0683	.0204	6
	<i>Diff.</i>	0.0067	0.0273	6
Spring	Right	.126	.0337	10
	Left	.0970	.0275	10
	<i>Diff</i>	-0.029	0.0289	10
Spring+Vibration	Right	.118	.0541	10
	Left	.103	.0231	10
	<i>Diff</i>	-0.015	0.051	10
Vibration	Right	.0810	.0119	10
	Left	.0990	.0191	10
	<i>Diff</i>	0.018	0.0155	10

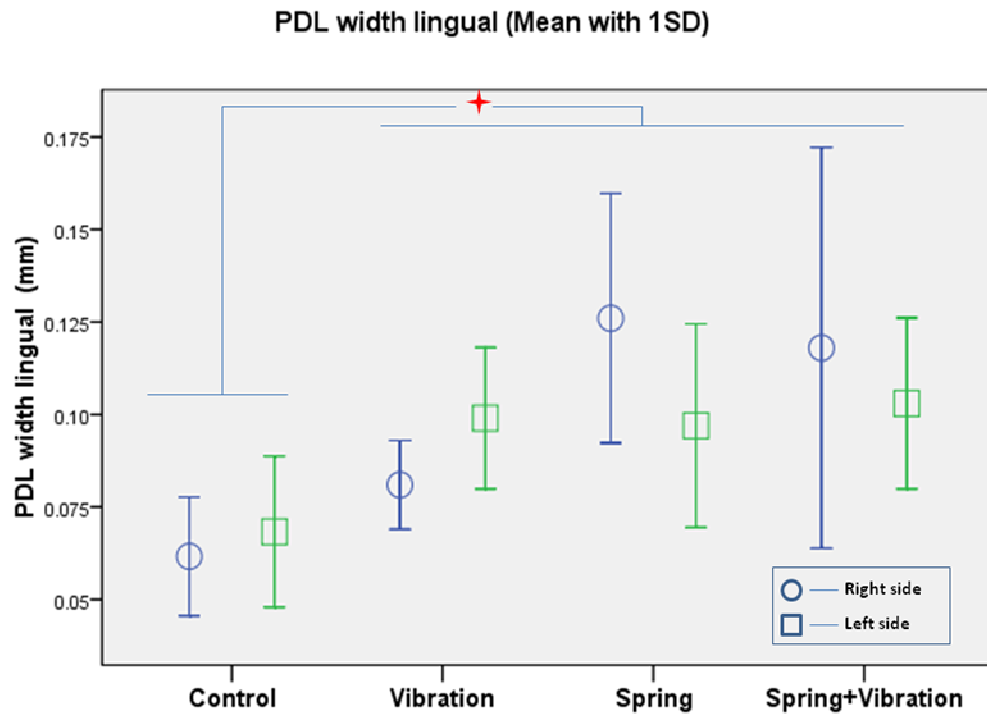


Figure 30: Graph depicting width of lingual PDL of mesio Buccal root on 1st molar for left and right sides in each group. Data were presented as mean \pm 1SD.

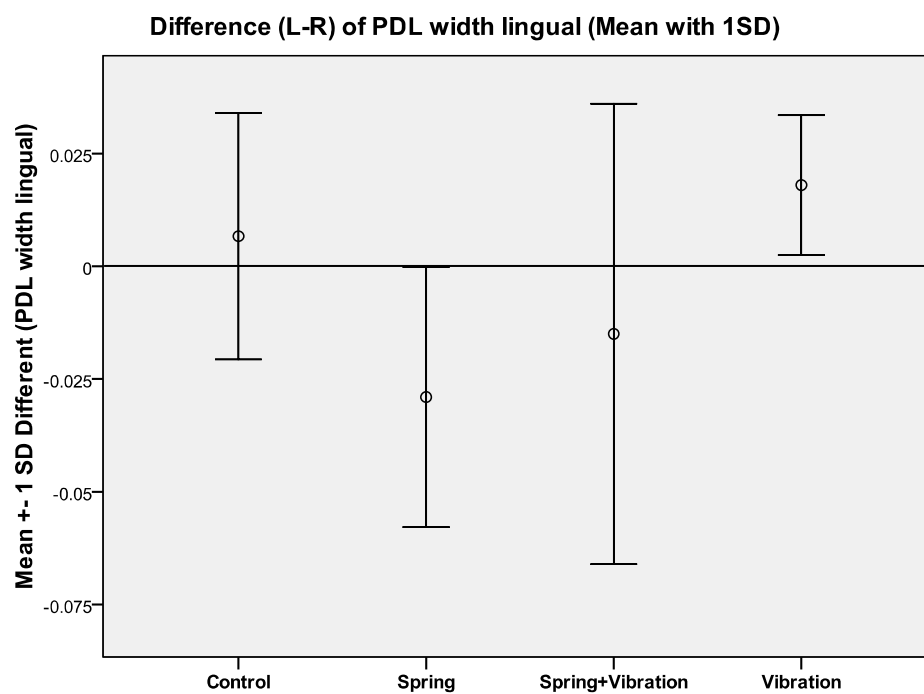


Figure 31. Graph depicting PDL width lingual of tooth differences between left side and right side (L-R) for each group. Data were presented as mean \pm 1SD.

6. Midskull to lingual plate (mm)

A statistically significant ($p < 0.05$) treatment effect was observed between the vibration group and the other three groups.

Table 11: Results of Midskull to lingual plate

	Side	Mean	Std. Deviation	N
Control	Right	1.733	.0582	6
	Left	1.721	.0591	6
	<i>Diff</i>	<i>0.0117</i>	<i>0.0445</i>	6
Spring	Right	1.759	.0517	10
	Left	1.773	.0742	10
	<i>Diff</i>	<i>0.014</i>	<i>0.0652</i>	10
Spring+Vibration	Right	1.756	.0514	10
	Left	1.799	.0392	10
	<i>Diff</i>	<i>0.043</i>	<i>0.0501</i>	10
Vibration	Right	1.671	.0321	10
	Left	1.666	.0359	10
	<i>Diff</i>	<i>-0.005</i>	<i>0.0417</i>	10

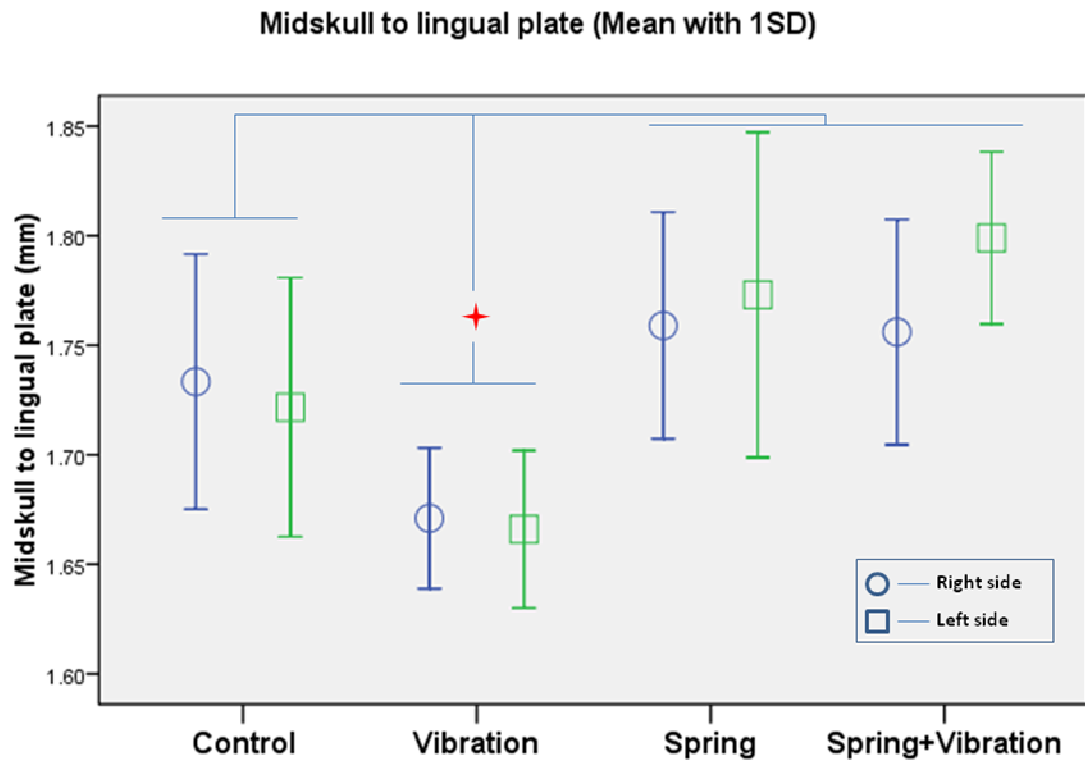


Figure 32: Graph depicting length from Midskull to lingual portion of 1st molar mesio-buccal root PDL for left and right sides in each group. Data were presented as mean \pm 1SD. (p-value <0.05).

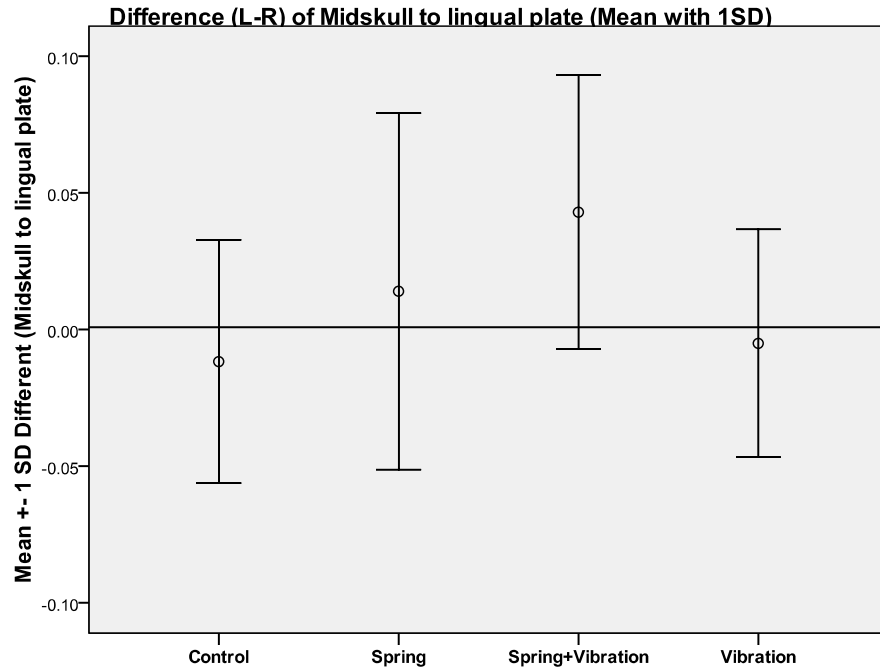


Figure 33. Graph depicting Mid-skull / lingual plate differences between left side and right side (L-R) for each group. Data were presented as mean \pm 1SD.

CHAPTER V: DISCUSSION

Animal Species

Researchers that study orthodontic tooth movement (OTM) in animals generally use rats as their model.^{37,40,48,49,50} The ability to use mice rather than rats is of interest mainly due to the array of knockout mice available for experimental testing and the relevance these knockouts have for understanding physiology. The difficulty of using mice rather than rats is their size. Simply said, it is easier to insert an orthodontic appliance and vibrate molar in a rat than a mouse. In addition, it is more challenging to make physiological measurements in mice. However, our attempt to provide a protocol to study OTM and vibration in mice is valuable because it may allow using knockout mice to test an almost unlimited numbers of very interesting hypotheses.⁴

Experimental OTM Models

1. Appliances

To induce OTM, several different types of devices have been used. Studies have used a closed coil spring that is attached to the first molar and incisor.^{7,40,50} The drawback of this model is that the incisors are used as an anchor that could be relatively moved and fractured or quickly worn out during chewing function. Additionally, the closed coil spring may not provide continual and consistent force over time. In our experience, once the spring was placed it was quite possible for food (mouse chew) to be caught in the individual coils, which made the coil less effective.

In order to achieve our study goal of comparing the effects of vibration versus non-vibration on orthodontic tooth movement, it is necessary to have a carefully designed

experimental protocol. In this regard, if using the coil spring to move teeth, then two separate coil springs will be individually engaged between the 1st molars and incisors bilaterally. Our pilot trial showed it is almost impossible to keep the force levels exactly the same between the two sides, hence an error of unequal loading will be introduced. To offset the potential for unequal loading, Nishimura et al (2008) used an expansion spring that was retained by its own force and delivered 12.8g of force to the 1st molars in rats.³⁷ Therefore, we decided to use Nishimura's expansion spring model, for two reasons: (1) the spring appliance delivers two equal and opposite forces on the bilateral 1st molars, avoiding the introduction of the error of loading force; (2) the spring appliance has the least possibility of being distorted by chewing and caught by food. However rather than have the spring be retained under its own force, we bonded the terminal curve end of the spring to the first molars. This is based on our prior experience with this spring appliance. We found that without bonding, the retention of the spring appliance in place could not be guaranteed for more than two weeks, which was not long enough for the current study.

2. Force magnitude

Forces used to induce experimental OTM have ranged from 5g to 57g^{7,37,40,50,51}. In our study, NiTi expansion springs were designed and specially manufactured by G&H wire company who calibrated the initial force delivery to be 20 grams. Two studies used a similar expansion spring. Nishimura³⁷ and Hou⁵¹ reported forces of 12.8g on a NiTi spring and 57g on a steel spring, respectively. We decided to use 20g NiTi springs for two reasons: (1) 20 grams of force (larger than 12.8g as reported by Nishimura) should be able to cause a definite hyalinization on which the vibration is targeting. Producing a

hyalinization area was important in order to test our hypothesis that mechanical vibration can reduce the degree of hyalinization due to collapse of blood vessels in the compressed PDL during OTM; (2) our experience has shown that 20 grams of force would gain much better self retention of the spring appliance than using lower forces such as Nishimura's 12.8g.

Vibration frequency

The range of frequency used in the literature varies from 1Hz to 61Hz (Nishimura (61Hz), Christiansen (45Hz), Rubin (30Hz), Darendeliler (30Hz, 15Hz), Stark and Sinclair (25Hz), and Warden and Turner (1Hz, 5Hz, 10Hz, 20Hz, and 30Hz)). A frequency of 4Hz was used in the current study. Warden and Turner⁵² studied ulnar vibration in C57BL/6 mice and found that cortical bone adaptation to mechanical loading increased with increasing loading frequency up to 5-10Hz. In contrast, Mcleod and Rubin¹⁶ predicted that a range of 15-30Hz will provide a maximum osteogenic response. Additionally, Mcleod and Rubin¹⁶ noted that strain magnitudes (force) that normally causes resorption when associated with a frequency of 1Hz, can cause new bone formation at 15Hz. . Christiansen³⁸ suggested that it may be possible that some frequencies produce almost no osteogenic response, despite positive results at lower and higher frequencies. Given the success of the previously mentioned authors in demonstrating an increase in the rate of bone formation or OTM at frequencies ranging from 5-61Hz, selecting 4Hz for the current study was certainly comparable considering the animal model used for testing.

Vibration duration

There is little published evidence that would provide guidance for selection of optimum duration and timing of vibration episodes. Whole body vibration studies have applied vibration every day for 10-15 minutes.^{38,39} Orthodontic tooth movement studies have ranged from applying vibration once a week to every day.^{37,41} Orthopedic studies have demonstrated that applying vibration 5mins/day every three days is an effective regimen for ulna, tibia, and femur.⁵² Due to the lack of a standardized approach, we chose to apply vibration at 5mins/day every three days for two reasons. First, Warden and Turner⁵² have shown success in their orthopedic studies, and secondly, animal care regulations required that mice needed three days of recovery after they were anesthetized. Ketamine and Xylazine were delivered as an intra-peritoneal injection, which usually takes 2-3 days to be metabolized. Unfortunately it was also not possible to deliver isoflurane as an inhalant anesthetic because we were working in the oral cavity. Given the regulations that had to be met and the reported evidence from the literature, applying vibration for 5mins/day every three days was deemed to be an effective protocol.

Intra-examiner variability

It is important that examiners can reliably exam the outcome variables selected for the study. Intra-examiner variability was acceptable for most outcomes. However, two exceptions were found: (1) coronal view, the measure of the length of the root from a line

bisecting the buccal and lingual CEJ's through the long axis of the root to the most apical portion of the root. (2) axial view, the width of the palatal suture measured at the position of the palatal root on the maxillary first molar. The following reasons may have accounted for the lack of reliability: (1) insufficient image quality to determine parameters; (2) the sharp and clear boundaries of bony structures are based on bone mineral density. Bone density is low (not yet mineralized) during the modeling process of OTM, thus rendering the definition of boundaries extremely difficult.

Differences of OTM between groups and between sides

In this study, a protocol of using micro CT technique to evaluate the orthodontic tooth movement under mechanical vibration was developed. Five variables (2 angular and 3 linear) were constructed on the coronal view and 6 variables (1 angular and 5 linear) on the axial view. By their definitions, the variables were determined to represent tooth positions except for the midpalatal suture, however they were not equally accurate and reliable. Some variables were hard to measure precisely on the CT image due to the image quality issue and lack of resolution. For example, distance "Midskull/CEJ" is well defined. However, because the expansion spring was left in place to avoid possible relapse of tooth movement during micro CT scanning, the image of the metal spring overlapped the CEJ. This made identifying the appropriate point along the CEJ difficult, affects the variable's accuracy and reliability. Assessing the reliability of the various variables, although beyond the scope of this study, could be valuable for further studies. Clearly, most variables used in this study were associated with substantial variability. This could have masked subtle differences that may have existed between treatment

groups and between sides within treatment groups. Potential causes of the high variable are discussed in limitations (below). The split mouth design is based on the high level of symmetry of features observed on both sides of the jaw. To assess differences that occur on both sides of the jaw, it is convenient to subtract the measurement of the right side from the same measurement on the left side. If the difference (L-R) is a positive “+” number, it is indicative of the left side 1st molar moving more than the right side molar, and vice versa. Theoretically, the difference (L-R) in the control group should be zero, since both sides received no treatment. The spring group should also be zero, since the applied forces were equal on both sides. The vibration group would also most likely lie near the zero difference, although there may be a slight tendency in either the positive or negative direction. In the spring/vibration group, it was expected that the distance or angle measured on the left side would be bigger than that on the right side. For instance, the variable “Midskull / CEJ” showed that the mean difference (L-R) of the spring/vibration group was positive, indicating that the left side had a tendency to tip more than the right side.

The present study failed to show statistical significance for the primary and most of secondary outcome variables. However, the study showed a strong overall treatment effect due to spring forces. Mice that were exposed to spring force or spring force in combination with vibration exhibited, as expected, significantly more tooth movement or tipping than animals in the control group or those who received vibration alone.

Root Resorption

Root resorption is a major concern in orthodontic treatment today. In an attempt to identify root resorption on the μ CT scans, the length of the mesio-buccal root of the

first molar was measured on both sides of the jaw. The results showed that the root length in the vibration group was significantly smaller ($p < 0.05$) than in the other three groups. No significant difference existed between the control, spring, and spring/vibration groups. Based on the theoretical consideration, the vibration and control groups were expected to have similar root lengths. In contrast, shorter roots were expected to occur in both groups that were exposed to spring forces. There is currently no adequate explanation for the vibration-induced root resorption. Possible reasons include the difficulty/error in locating the most apical portion of the tooth due to its irregularity, or pinpointing the exact location of CEJ's. It may be possible that a more conclusive answer can be obtained from histology and histomorphometry analyses.

Limitations and Future Directions

This was a pilot study to evaluate the usefulness of a murine model for the study of vibration as an adjunct to spring forces in OTM. Several aspects of the protocol deserve special consideration.

1. Spring insertion and retention

Given the size of mouse molars, it was very difficult to clean the tooth surface and place the adhesive on the spring and 1st molar only. There was a chance that the adhesive spread onto the occlusal surface of the 1st or 2nd molars or interproximately, bonding the 2nd molar to the 1st molar and spring, which potentially introduced another factor into the force system.

2. Vibration

Due to anesthesia requirements and recovery time the mice were vibrated once every three days. Although there is little to no evidence currently in the literature, it would be interesting to determine if more frequent vibration would increase OTM.

3. CT scan and reconstruction

A big limitation of this study is that it was not possible to take baseline micro CT scans before force and vibration application. Micro CT uses high dose of radiation that was determined to be lethal if used repeatedly. A new generation μ CT machine that uses a lower dose radiation would allow taking μ CT images of living mice. Repeated μ CT images would be a definite improvement, possibly also leading to improved measurement quality.

Another crucial step was the method of taking the μ CT scan. When the specimen was placed on the holder, it was necessary to assure no motion to prevent blurring of the image. We attempted to take the scan with mouse skull affixed inside a capped plastic cylinder with and without liquid, but found the cylinder affected the image quality. Movement could affect the reconstructed images by compromising the ability to define landmarks due to pixilation. The scanning process will be reassessed since this is the basis for obtaining a reliable image to complete the measurements.

4. CT image measurement

Once the image was reconstructed, it was necessary to orientate the image for obtaining proper coronal and axial views. Since the force was applied only to the teeth

across the midpalatal suture, it was assumed that the cranial structures would provide sufficient stationary landmarks as reference for making measurements. Obtaining an absolutely symmetrical view is difficult when aligning the mesio-buccal root. The alignment was improved when it was based on the palatal root of the 1st molar. It is important to symmetrically align the left and right sides when obtaining the coronal and axial images. If the symmetry is off, the measurements will reflect the error. Although an honest effort was made to achieve symmetrical views, there may be errors associated with aligning and measuring the coronal and axial views.

In conclusion, this pilot study attempted to produce a murine protocol of using micro CT to study orthodontic tooth movement under mechanical vibration. A specially designed expansion spring was introduced to generate orthodontic tooth movement, upon which mechanical vibration (4Hz frequency, 20micron displacement, 5min/session/3days) was imposed during a 21 day study period. Significant orthodontic tooth movement was observed in the spring and spring/vibration groups versus the control and vibration alone groups. Differences between spring and spring/vibration groups as targeted were not identified statistically, however an overall tendency was observed demonstrating that the vibrated side in the spring/vibration group had increased OTM when compared to its contra-lateral non-vibrated side. Further efforts need to be made to improve the protocol, especially with respect to measurement accuracy and selection of a reliable primary outcome variable.

CHAPTER VI: BIBLIOGRAPHY

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