2018

# Minimally Invasive Accelerated Orthodontic Techniques: A Clinical, Radiological, and Histological Comparison on a Rat Model

Sausha Toghranegar
*Nova Southeastern University*

# *Minimally Invasive Accelerated Orthodontic Techniques:*

## *A Clinical, Radiological, and Histological Comparison on a Rat Model*

Sausha Toghranegar DMD

Saynur Vardar-Sengul DDS PhD

Shiva Khatami DDS PhD

Umadevi Kandalam PhD

Health Professions Division

College of Dental Medicine

Department of Periodontology

A Thesis Presented to the Faculty of the College of Dental Medicine of Nova Southeastern

University in Partial Fulfillment of the Requirements for the Degree of Master of Science

September 2018

*Minimally Invasive Accelerated Orthodontic Techniques:*

*A Clinical, Radiological, and Histological Comparison on a Rat Model*

By

Sausha Toghranegar

A thesis submitted to the College of Dental Medicine of Nova Southeastern University in partial fulfillment of the requirements for the degree of Master of Science , College of Dental Medicine, Department of Periodontology, Nova Southeastern University September 2018. Approved as to style and content by:

APPROVED BY:_____

Name of Mentor Date

APPROVED BY:_____

Name of Committee Member Date

APPROVED BY:_____

Name of Committee Member Date

APPROVED BY:_____

Dean: Date


Health Professions Division

Department of Periodontology

College of Dental Medicine

STUDENT NAME: Sausha Toghranegar D.M.D.

STUDENT E-MAIL ADDRESS: st641@nova.edu

STUDENT TELEPHONE NUMBER: (813) 451-4667

COURSE DESCRIPTION: Master of Science in Dentistry with specialization in postgraduate Periodontics

TITLE OF SUBMISSION: Minimally Invasive Accelerated Orthodontic Techniques: A Clinical, Radiological, and Histological Comparison on a Rat Model

DATE SUBMITTED: September 2018

I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.S. degree and for this assignment.

STUDENT SIGNATURE:_____

# ACKNOWLEDGMENTS

I would like to thank my advisor, Saynur Vardar-Sengul, for her many years of thoughtful, patient guidance and support. Thanks are also due to Gabriella Ambrose, Abelardo Daya and John T. Ritter. Together their friendship and selfless contribution to my professional development have been invaluable and will forever be appreciated. I would also like to extend my gratitude to the members of my committee, Shiva Khatami and Umadevi Kandalam, for their helpful comments and suggestions on all stages of this project. I want to thank Piezosurgery, Propel, Lenyosys, and Henry Schein for providing materials. Special thanks to all those whose support and friendship helped me to stay focused on this project and provided me with the encouragement to continue when the going got tough.

## Abstract

**Background:** Accelerated orthodontics encompasses a group of techniques designed to facilitate the faster movement of teeth. Many techniques developed are designed to cause a controlled injury to the cortex of the bone resulting in a transient osteopenia, also known as the regional acceleratory phenomena (RAP). Little research has been done to compare these techniques and describe their effects on the periodontium. **Objective:** To clinically, histologically, and radiographically compare several minimally invasive techniques for inducing accelerated orthodontics. **Methods:** Sixty 8-9-week-old male Sprague Dawley rats were used for this investigation. An orthodontic device consisting of a 50g NiTi closed coil spring was applied to allow for the mesial movement of the upper left first molar. Rats were divided into 4 groups, one control (n=15) and 3 minimally invasive accelerated orthodontic interventions consisting of Piezocision (n=15), Propel (n=15), and pulsed electromagnetic fields (PEMF) (n=15) were included. Five rats from each group were euthanized for histology at 3 time points from baseline at Day 7, Day 21 and Day 49. Histomorphometric and descriptive analysis were performed using axial cross-sectional slides of the mid-root region. For clinical analysis, the distance from incisors to test molars were measured with digital calipers at baseline and post-treatment time points. Cone beam computed tomography and micro computed tomography were performed. **Results:** Bone density was found lower in the Piezocision and Propel groups at day 21, and the periodontium re-organizes by day 49. Piezocision had statistically significant reductions in histologic and radiographic bone density ($p<0.05$). **Conclusion:** Decortication techniques, such as Piezocision and Propel, resulted in more osteopenia and tooth movement. Utilization of decortication may facilitate tooth movement through the alveolus to provide an accelerated and safe movement.

Pulsed electromagnetic fields may demonstrate the potential for regulating bone metabolism without decreases in bone density.

## CONTENT

# Introduction

Accelerated orthodontics is a group of techniques that are used to facilitate the faster movement of teeth to achieve relatively shorter treatment times. Reducing treatment times could be a significant factor in providing ideal treatment to the adult population. More adults are wearing braces to align their teeth for better function and esthetics. The benefits of strategically placing teeth in appropriate maintainable positions can vastly improve the long-term prognosis of both minor and complex treatment (Ainamo 1972, Yared et al. 2006, Zhang et al. 2017)). Some of these minor applications of orthodontics include up-righting of tilted teeth, de-rotation of teeth, space closure, orthodontic extrusion, etc. In certain cases, adult patients can benefit from comprehensive full arch orthodontic movements. For example, in cases of posterior bite collapse, tooth migration may limit restorative space and move teeth out of ideal restorative positions. In these cases, ideal treatment often includes a comprehensive orthodontic plan. Though most adult patient treatment plans often include some level of orthodontics, it is still considered to be poorly accepted by both patients and clinicians. Some potential reasons for poor treatment acceptance include time, esthetics, and cost of adult orthodontic treatment (Uribe et al. 2014, Varela and Garcia 1995). In both minor/localized and comprehensive treatment, accelerated orthodontics can increase the speed and of movement and potentially reduce treatment time (Uribe et al. 2014). This may increase patient compliance for ideal treatment and improve the quality of patient care.

## Biomechanics of Tooth Movement

Due to the nature of the bone, orthodontic movement of adult teeth requires extended amounts of time. This reasoning for the relative increased treatment time of adult teeth is the result of several factors including psychology, bone density, vascularity, systemic status, and epigenetic stability (Masella and Meister 2006, Rosa et al. 2015, Skidmore et al. 2006). Understanding the biomechanics of tooth movement can shed light on how challenges with treatment time can be overcome.

Bone is an organic matrix consisting of collagen amalgamated with crystalline hydroxyapatite. Although the relative mineralization and non-organic ratio of contents within bone have a significant impact on the physical properties of bone, the cellular and vascular component of bone plays a potential role in the movement of teeth through the alveolus (Murshid 2017, Sodek and Mckee 2000). The interaction of pressure and tension within the periodontal environment is a well-accepted model for describing the macro and microbiological reactions when a force vector is applied (Feller et al. 2015). The pressure side results in compression of the periodontal ligament (PDL) space, hyalinization, and osteoclastic resorption. The vessels within the periodontal ligament space are compressed and result in fluctuations in gas, fluid, and cellular flow. Additionally, the vessel compression can result in localized sites of necrosis which will require cleaning-up or resorption, which is later replaced. With the localized compressed vessels comes ischemia and ischemic necrosis. Vascular endothelial growth factor (VEGF) has been shown to be upregulated in these regions to facilitate the growth of new vessels and re-establishment of vascularity (Miyagawa et al. 2009). The catabolic orchestration causes an altered pH and cytokine release resulting in a paracrine and/or autocrine cellular signaling cascade. The signals then bring about a series of reactions to recruit cells to resorb the hyalinized areas. This creates a path in

which the force can now move in the direction of the vector with a decompressed vascular supply. Without excessive orthodontic forces, the amount of bone necrosis is controllable through the remodeling process. If excessive forces are utilized, then necrosis can become extensive leading to significant amount of undermining resorption and root resorption (Deguchi et al. 2014, Masella and Meister 2006). This has the potential to pathologically damage the tooth, attachment, and delay tooth movement.

The molecular and cellular contents are in flux during strain to the PDL. Compression hydrodynamics forces molecules throughout the PDL space allowing activation of matrix metalloproteinases (MMPs), serine proteases, aspartate proteases, and cysteine proteases. These enzymes begin to digest the extracellular matrix and form a less dense, more elastic network (Krishnan and Davidovitch 2006). This allows a reduction in pressure while also exposing new molecules and pathways for these molecules to reach fibroblasts and mesenchymal cells. The cells also directly respond to this mechanical strain. Mechanical forces in the surrounding extracellular matrix are directed on the cells, creating alterations to the cell membrane and the cytoskeleton. These changes can set off a chain reaction leading to increased receptor activator of nuclear factor-$\kappa\beta$ (RANK) and interleukin 1β (IL-1β) expression. The monocyte/macrophage lineage gives rise to osteoclasts as the RANK ligand (RANKL)/ osteopotegrin (OPG) ratio increases within the environment (Feller et al. 2015, Murshid 2017).

At the same time, the tension side is undergoing a similar yet opposite series of events. The periodontal ligament fibers are being stretched. The mechanical strain of tension results in a very different cascade of cellular responses then does the pressurized PDL region. Mesenchymal cells within the alveolar bone respond to the tension strain by upregulating mitogen-activated protein kinase (MAPK) intracellular signaling pathway. This has been shown to upregulate the transcription factor Runx-2, which plays a critical role in regulating the maturation of osteogenic

cells into osteoblasts (Feller et al 2015). Other MAPK pathways via ERK 1/2, JNK, or p38 have all been indicated in osteoblast maturation and function in relation to mechanical amount of mechanical strain (Zhu et al. 2008). For example, a p38 cascade can upregulate RANKL production by osteoblasts resulting in an increased RANKL/OPG ratio, hence increased bone resorption and remodeling. The mechanical tensing formed on the cells can alter the diameter of the cellular membrane channels (Huang et al. 2004). This would affect ion and molecular flow potentially altering the activity of the cells. The tensing of the fibers also dissipates molecular flow through the extracellular space analogous to the compression side. The main difference being the content of the materials being shifted around by this hydrodynamic flow. Nonetheless, this sets off a signaling cascade to recruit fibroblasts and osteoblasts to build bone and re-organize the periodontal ligament.

Bone is known to have a biomechanical response to forces, especially alveolar bone. The mere presence or absences of teeth dictates its existence. Understanding that the absence of teeth or implants result in the loss of alveolar bone, then their presence may have unique factors that sustain the presence of bone (Chen et al. 2013, Cowin et al. 1991, Esther 2010, Zhu et al. 2008). One viable explanation is the vascular supply of the periodontal ligament. This rich vascular source can breathe nutrients and vitality to the surrounding alveolar bone. However,, dental implants do not have vascular PDL, yet the alveolar bone remains. Another explanation is that the forces applied to the tooth or implant generate a mechanical signal that is reciprocated with a biochemical response to sustain and adapt the alveolar bone(Chen et al. 2015, Cowin et al. 1991, Feller et al. 2015, Krishnan and Davidovitch 2006). By simply stretching and compressing PDL fibroblasts, genetic regulation of collagen synthesis and matrix metalloproteinase (MMP)-2 can be seen[19]. When a functional load is applied to teeth, forces are transmitted to the underlying bone and PDL. This force applies strain to the collagen fibers in the bone and PDL. The shearing

strain on the collagen fibers and fluid results in a deformation that shifts the location of the charged regions. This generates areas of electronegative and electropositive charges. In turn, this generates an electrical potential in the region. The electrical potential can then affect cells and cellular signaling (Rubin et al. 1993). Cellular responses to energy have been documented by in-vitro and in-vivo studies (Burger and Klein-Nulen 1999, Cowin et al. 1991, Rubin and Lanyon 1984). Although the focus has much been on the mechanical forces causing the electrochemical based effects, the nature of electrical energy's potential to alter the biochemistry of the periodontium has not been forgotten (Dogru et al. 2014). Cytokine production and chemotaxis have been shown to be affected solely by electromagnetic energy input without direct influence from biochemical energy sources (Dogru et al. 2014, Shupak et al. 2003, Stark and Sinclair 1987). An important example demonstrates RANKL production to fluctuate by the application of electromagnetic radiation (Zhang et al. 2017). This suggests that the energy generated by stresses applied to bone and collagen can directly play a vital role in the functions of cells. The cells localized in the alveolar bone, such as mesenchymal cells, fibroblasts, and endothelial cells, will have a reaction to these forces that can affect bone/collagen metabolism (Shupak et al. 2003).

Additionally, the deformation caused by stresses to the alveolar bone generates fluctuations in the hydrodynamic flow in bone and surrounding tissues (Will 2016, Rosa et al. 2015, Ramani-Mohan et al. 2017). This facilitates the movement of fluids within the localized region including the PDL space, canaliculi system, and marrow spaces. When a force is applied to a single tooth, the localized alveolar bone is compressed. The fluid within this region is hydraulically moved creating shear stress. This results in a cascade of mechanosensory changes . It is suggested that high frequency, low level fluid-based shear stress is a key factor in bone remodeling (Rosa et al. 2015). Some of the effects are electrochemically induced, as previously mentioned. Another is the relative gradient changes resulting from fluids moving around within

the localized system. Cytokine latent fluid within this region is forced outwards and encounters cells that have now been introduced to a higher level of cytokines and nutrients. These distant cells, who have just been introduced to a bounty of cell altering molecules, have the ability to upregulate, as well as down regulate, certain cell signalers (Rosa et al. 2015). Once the force on the tooth is relieved, the elastic deformation rebounds allowing the fluid, which now has an altered concentration of cytokines and cells, to flow back to the location of the tooth. The cells in the bone and collagen surrounding the affected tooth are now exposed to an array of outsourced cytokines. This change in concentration of certain molecules results in a cellular response. Thus, the localized cells respond with the potential to alter the bone morphology surrounding the tooth. Osteoclasts can be induced by a possible influx in the presence of RANKL, followed by the induction of osteoblast formation via RANKL outflow or OPG inflow. The shear stress of the fluid flow can also directly be applied on cells. The strain on cells is enough to trigger morphological and functional changes (Chen et al. 2013, Huang et al. 2004). In tandem, these hydraulically induced changes can result in a cellular response aiding tooth movement.

**Piezoelectric Potential of Bone**

Another potential source for facilitating the cellular signaling takes into consideration the piezoelectric nature of bone. Much of the dental research on bone focuses on the biochemical and cellular properties. Bone is a solid and, thus, is subjected to the natural laws of physics that apply to solid objects. One physical property of bone that is of interest is its piezoelectric property (Donahue 2000, Ren et al. 2015, Marino and Gross 1989). The unique crystalline structure of piezoelectric objects allows them to either create energy upon being deformed or to deform upon application of an energy source. The classical example is quartz. When a voltage is applied to quartz, it will deform or change shape. On the other hand, if quartz is compressed manually, it

will generate a voltage. For example, the piezoelectric surgical instruments utilize ceramics and their piezoelectric property to generate ultrasonic vibrations of a surgical insert to cut hard tissue. The crystalline structure of quartz has a certain configuration of disbursed negatively and positively charged atoms creating a distinct neutralized charge. Upon compression, atoms shift and the polarization changes. This results in a potential difference and the formation of electrical energy. Bone has been shown to have piezoelectric properties (Ahn and Grodzinsky 2009, Marino and Gross 1989). Studies on dry bone were clearly able to note the piezoelectric property of bone. Contradicting results have been demonstrated in wet bone. As the water content increased, the polarizability of bone and collagen can increase but the piezoelectric potential is dampened (Ahn and Grodzinsky 2009, Netto and Zimmerman 1975). Even when the potential is reduced, the piezoelectric property is retained. A string of studies demonstrating similar results with wet versus dry bone began to reduce the viability of the piezoelectric effects on bone biophysics (Anderson and Eriksson 1970, Burger and Klein-Nulen 1999, Netto and Zimmerman 1975). Researchers argued that wet bone *in vitro* studies best represent what would likely happen *in vivo*, suggesting that the piezoelectricity within the body would be dampened possibly to a point of no affect. Other physical properties, such a fluid flow related shear stress and streaming potentials began to take center stage, shifting the scientific literature focus away from piezoelectricity (Ahn and Grodzinsky 2009, Anderson and Eriksson 1970, Netto and Zimmerman 1975, Marino and Gross 1989). More recent studies utilizing more advanced technology have begun to re-shift some focus to the potential piezoelectric properties of bone. For example, a more recent study utilizing a Piezo-response Force Microscope was able to demonstrate piezoelectric properties of both wet and dry human long bones (Halperin et al. 2004). Initial studies on dry bone indicate that it is not the inorganic hydroxyapatite component responsible for the bulk of the piezoelectric effect, but rather the collagen(Ahn and Grodzinsky 2009). Upon stress of the collagen fibers, the charge

carrying portions of the macro molecule are displaced from the inside to the surface resulting in the potential being formed (Stroe 2013). The deformation that occurs along the collagen fibrils is the deformation that results in piezoelectricity. The hydration around these collagen macromolecules has been indicated in the reduction of the piezoelectric effect. But the localized positioning of water molecules can vary depending on the type of bone. Higher amounts of mineralized hydroxyapatite can aid in shielding the collagen from water and reduces the dampening of piezoelectric properties (Marzec et al. 1996). For example, cortical in vivo may be more piezoelectric then cancellous bone. Nonetheless, evidence exists to suggest bone has a piezoelectric potential.

The summation of all these potential sources for bone modification can be used to control the direction of tooth movement. Utilizing a controlled vector, one side has an upregulation of osteoblasts and the opposite has an osteoclastic environment. The osteoblast side will have a resulting increase in new bone formation and calcification, and the osteoclastic side will have resorption of bone. This creates a path of which the tooth can migrate through demineralized connective tissue as bone is laid down behind. Once this vector is halted, bone is remodeled and ideally the periodontium is stabilized in the absence of insulting inflammation, such as that derived from pathogenic bacteria (Will 2016, Masella and Meister 2006).

## Adult Orthodontic Treatment

Although younger adolescent patients are often thought of as the main recipients of orthodontic treatment, the need and want for orthodontics in adults is growing. As this need grows, so does the awareness of the differences in the bone's ability to allow tooth movement in younger patients versus adults. Younger adolescent patients have not completed growth. There have been documented changes in bone mineralization, collagen matrix, and non-collagenous

protein levels in adults that makes tooth movement more difficult (Sodek 2000, Wang et al. 2002). The adolescent alveolar bone has the luxury of an upregulation of anabolic related hormones. The collagen network is also more likely less disturbed and can better respond to the turnover process (Wang et al. 2002). Thus, the orchestration of bone remodeling can occur much more smoothly and allow for rapid turnover and faster tooth movement. Additionally, the bone mineral content of adults has likely gone under primary and secondary mineralization. The higher density of bone requires greater effort on behalf of the osteoclasts to turn over to allow a tooth to advance in the vector of the applied force. This requires a greater recruitment of osteoclast mediators, osteoclasts, and their by-products. This higher RANKL environment can also result in the production of other inflammatory mediators, such as IL-1b and TNF-α (Feller et al. 2015). Adult patients already have a higher potential for inflammatory diseases including but not limited to periodontitis (Weyand et al. 2014). Despite the potential for excessive inflammatory destruction, comprehensive orthodontic treatment has proven to be effective and safe in adults without resulting in any significant periodontal destruction ( Charavet et al. 2016, Choo et al. 2011, Wilcko 2013). Thus, if additional pathological inflammation is controlled, then excessive tissue breakdown beyond regeneration or repair does not occur. The local “clean” inflammation that occurs by orthodontic tooth movement can occur in adults without damage to the tooth or the periodontium. On the other hand, this pressure-induced inflammation has been known to result in significant tooth resorption and ankyloses (Deguchi et al. 2014). This pathological inflammatory response has been linked to excessive forces during tooth movement. The pathology behind this phenomenon has been suggested to be the result of too much pressure resulting in undue necrosis. Since adults have a higher density bone, the direction of the vector often results in greater potential resistance and pressure. This is a major reason that typical orthodontic

movement in adults must be performed with lower forces and hence a slower rate. This may result in relatively extensive treatment times for adult orthodontic patients (Wang et al. 2002).

It has also been shown that adult patients have less compliance with orthodontics, which in turn elongates the treatment time (Skidmore et al. 2006, Melo et al. 2013). As treatment time becomes longer, the adult patients will likely have even less compliance, resulting in a feedback loop of compliance issues. The longer braces are worn the more issues come to rise. Braces provide a plaque trap that can cause many pathological effects on teeth and periodontal tissues. The plaque that accumulates can cause gingival inflammation, which could lead to more serious conditions of the periodontium (Addy et al. 1982). Gingival overgrowth and fibrosis due to this inflammation may require surgical intervention to correct. Plaque can also cause demineralization of enamel and lead to white spot lesions on the teeth that may require additional treatment to correct (Boyd and Baumrind 1992). Conventional orthodontic treatment requires the teeth to be moved at a certain rate to allow for just the right amount of force. If these forces are increased in an attempt to move teeth faster, bone loss and tooth resorption could occur (Deguchi et al. 2014). However, with the accelerated orthodontic techniques, teeth can move faster without the need for pathological forces (Alikhani et al. 2013, Dibart et al 2011, Shenava et al. 2014). With treatment time reduced, patients have a better chance of avoiding the complications of plaque accumulation around the braces and brackets

Cost and esthetics of orthodontics has also been considered to be a limiting factor in treatment plan acceptance in adults. Chair time has highly been considered a valuable measure of a clinician's worth. If there was a way to reduce the amount of chair time, patients would take up, then it may conceivable to possibly reduce the cost to the patient. Typically, a patient is charged a single fee for orthodontic treatment and this fee will not fluctuate based off the amount of time spent in treatment once treatment commences. Accelerated orthodontics could result in

quicker movement of teeth and may result in fewer visits for the patient. If a clinician now has finished an orthodontic case in 1 year instead of the anticipated 2 years, this frees up chair time for a year in which another patient can be seen. Survey studies have indicated that parents would be willing to pay up to 20% additional money to speed up the treatment of their child's orthodontic treatment (Uribe et al. 2014). Thus, it may be conceivable to assume that although the innate cost of orthodontics may limit treatment plan acceptance, the ability to reduce time outweighs potential cost limitations. This may suggest patients have a greater concern for time of treatment than the cost.

The esthetics may also be a realm of concern for patients. The stereotypical orthodontic patient is a teenager, not a grown adult. The presence of orthodontic appliances can potentially affect the self-esteem, body image and social life of an adult patient (Varela and Garcia 1995). Others that the patient may encounter, such as friends and employers, may have altered psychosocial responses as well. The improvements in body image and self-esteem potentially indicated, mainly are seen after treatment is complete (Varela and Garcia 1995). In addition to esthetics, the lengthy nature of adult orthodontics results in low patient acceptance. Esthetics is closely tied to time. Longer treatment times mean longer the patient remains in perceived unaesthetic orthodontic appliances. Thus, having a method to reduce treatment times, such as accelerated orthodontics, will also aid in alleviating a patient's apprehension of the esthetics and time of orthodontic treatment.

## Periodontics and Orthodontics

In addition to providing faster movement of teeth, these facilitated orthodontic techniques allow for a better collaboration between periodontists and orthodontists. This additive team dynamic allows for more comprehensive and ideal treatment for patients.

Orthodontic patients need to have periodontal inflammation and caries under control prior to progressing with treatment. From a periodontal and restorative stand point, having orthodontics can potentially place teeth in more maintainable positions (Joss-Vassalli et al. 2010). Reduction of crowding can facilitate the hygiene. Closure of slight diastemas, especially in the posterior, can reduce food traps and prevent possible impaction of debris subgingivally. Moving teeth in into a more favorable occlusion can prevent possible periodontal inflammatory exacerbation from secondary occlusal trauma (Ainamo 1975). For example, reducing a steep anterior overbite or closing an anterior open bite may provide proper occlusal forces in protrusive movements. Understanding the patient's periodontal, restorative, and orthodontic needs and concerns is key to ideally treating and preventing potential issues. Improper understanding of the soft and hard tissue biotype can result in possible recession if teeth are mobilized outside of the alveolar housing. Recession on the facial, especially on the lower anteriors and bicuspids is a very common sequela of orthodontic tooth movement (Yared et al. 2006, Joss-Vassalli et al. 2010). Recession due partially to orthodontic movement could be prevented in many cases with a proper periodontal assessment prior to orthodontics. On the other hand, just having a diagnosis of thin tissue does not necessitate needing pre-orthodontic grafting. If the patient has signs of inflammation and orthodontic tooth movement is planned in the direction of thin tissue, often facially on the lower anteriors, then grafting would be indicated (Dibart et al. 2013, Wilcko et al. 2003). Thus, clinicians of all specialties need to be aware of all aspects of treatment. Without understanding the orthodontic plan, a periodontist could not fully evaluate sites that will benefit from pre-orthodontic treatment. Accelerated orthodontics requires a complete understanding of the planned orthodontic treatment plan. Stages of bonding and movements must be understood to properly plan the location and timing of corticotomies in addition to knowing areas that would benefit from grafting. The amount of time for which the acceleration is considered to work varies

amongst techniques, and thus must carefully be coordinated based on timing of movements (Dibart et al. 2011). If a significant leveling and aligning is planned prior to closing large edentulous spaces, then it is possible that accelerated orthodontics may need to be performed multiple times. Knowing the exact teeth being moved and which are designed to be anchors is key (Dibart et al. 2011). For example, a molar may be designated to be anchorage for moving neighboring teeth. If corticotomies to speed the movement are performed too close to the molar, then the relative anchorage could decrease and possibly lead to inadvertent movements. The orthodontist and surgeon must be aware of where corticotomies should be placed. Being unaware could result in improper use of the relative anchorage and cause teeth to move in unpredicted patterns. Knowing which teeth are now capable of faster movement can help the orthodontist plan adjustments accordingly. Thus, the periodontist and orthodontist are required to communicate their assessments and plan to accomplish accelerated orthodontics. This, in turn, results in ideal team treatment planning (Dibart et al. 2011).

## History of Accelerated Orthodontics and Periodontally Accelerated Osteogenic Orthodontics (PAOO)

The concept of accelerated orthodontics is not a new one. The concept of creating corticotomies to accelerate tooth movement has been documented in the year 1893 by L.C. Bryan[2]. Dr. Henrich Khole in 1959 suggested that the dense cortical plate can limit tooth movement. Cortical bone does not contain significant vascularity and has increased density. Thus, more time would be required to turn over the bone and allow for tooth movement. With this theory in mind, Henrich Khole (1959) suggested that decorticating and creating mobile blocks of bone containing the teeth to be moved would no longer limit the movement of the teeth. The thought was that the block of bone would move with the tooth rather than the tooth alone. In

1996, Thomas and William Wilcko made an observation that it was not the boney blocks migrating, but rather the corticotomy around the tooth was resulting in a regionally acceleratory phenomenon (RAP) (Wilcko and Wilcko 2009). The RAP effect was initially described by an orthopedist Dr. Harold M. Frost (1983). Basically, the RAP effect can be described as a localized healing response to a noxious stimuli or injury in the bone (Frost 1983). The neighboring hard and soft tissue change in order to help repair the neighboring injury. Thus, the Wilcko et al. 2003 reported that the controlled injury, or corticotomy, in the alveolar bone would result in a localized alteration, or osteopenia, that would facilitate tooth movement. With this concept, it became apparent that the amount and depth of the corticotomy did not need to be excessive. In other words, there was no need to remove so much bone as to create mobile blocks of bone. By simply removing an apico-coronal line of cortical bone interproximally on the facial, the surrounding bone would respond by a reduced density and allow for faster tooth movement. They were able to document many cases and several controlled studies to demonstrate the effectiveness of their modified corticotomy technique, and coined their technique Wilckodontics, also known as PAOO (Wilcko 2013) . They were able to demonstrate a nearly 2-fold reduction in treatment time utilizing their technique. In a controlled clinical study, Makki et al. 2014 compared similar mandibular crowding cases treated with conventional orthodontics against PAOO. They were able to demonstrate a statistically significant reduction in treatment time as well as significantly less relapse after ten years (Makki et al. 2014). Another major component of their procedure was the utilization of bone grafting onlayed over the corticotomy sites. The original technique did not require the use of a barrier membrane over the bone particulate. As the technique has evolved, several modifications in grafting material and membrane use have been suggested (Wilcko et al. 2015)

## Regional Acceleratory Phenomenon (RAP)

The RAP effect as mentioned before, is a key underlying mechanism for all these previously mentioned techniques. Dr. H.M Frost had developed the concept of the regional acceleratory phenomenon (Frost 1983). He noted that the surrounding bone was altered upon injury. This healing was not isolated to just the exact site of injury (Frost 1983)). Soft tissue injuries typically have significant vascular supply to initiate a healing response. Bone has limited vascularity, especially cortical bone. This results in limited cellular access and signaling. To bring sufficient cells to clean and rebuild, the neighboring soft tissue and relatively vascular cancellous bone needs to provide support. The access of cells to the cancellous bone is simplified by demineralizing neighboring cortical bone. This can be accomplished due to increase in inflammatory cells, which tend to help upregulate osteoclast maturation, chemotaxis, and activity. The mineralized cortical bone and denser cancellous bone undergo controlled demineralization. The breakdown of osseous density increases room for cell and vascular migration. The increased vascular access is coupled with an influx of progenitor cells from various sources, including the newly arriving perivascular progenitor cells. This helps the anabolic bone-remodeling phase via osteoblastic activity (Frost 1983, Sebaoun 2008). The help provided by neighboring tissues allow for proper healing of the osseous injury. This RAP effect results in a transient osteopenia, but eventually returns the bone quality to pre-injury levels. It begins 1 week after injury, peaks at 2 months, and returns to baseline at 4 months (Frost 1983). The Wilcko et al. (2003) described the RAP effect first time as the underlying cause for the transient increase in tooth movement following corticotomies.

The RAP effect can also be used to describe normal orthodontic tooth movement as well. The controlled injury would be the pressure necrosis that occurs upon compression of the

periodontal ligament, which creates a lag period of tooth movement. During this period, a process similar to the RAP effect occurs to allow for the transient influx of inflammatory cells and osteoclastogenesis. The amount of RAP effect is substantially greater with the placement of cortical injuries. This is reflected in the histological studies observing bone demineralization (Dibart et al. 2013). Clinically, it is demonstrated by the increased speed of tooth movement and decreased treatment times (Alikhani et al. 2013, Bemard 1990, Dibart et al. 2011, Wilcko et al. 2003).

## The Road to Minimally Invasive Accelerated Orthodontics

A common complaint amongst patient's and clinicians alike is the invasiveness of the classical corticotomy techniques, such as PAOO. A full thickness flap must be created on the arch involved and typically extends nearly the full length of the arch. The flap is reflected beyond the apex and involves incising through papillae. Reflecting a full thickness flap may result in loss of thin facial alveolar bone due to disruption of vital periosteum (Wood 1972). Wilcko et al. (2003) was able to demonstrate on a case series looking at post-operative cone beam computed tomography images that bone particulate graft was able to increase facial bone thickness. They suggest that their technique can increase the band of keratinized tissue. In cases of facial advancement of lower anteriors in areas of thin biotype, increasing keratinized tissue could be beneficial (Wilcko et al. 2003). Their statements could not be substantiated with any histological evidence that the visual soft tissue changes they saw was actually increased the amount of keratinized epithelium (Wilcko et al. 2015). Subsequent grafting can alleviate the issue of alveolar loss, but the amount of grafting further increases the complexity, cost, and time of the procedure. To perform the full thickness flap on nearly a whole arch, vital anatomy (i.e. mental nerve) needs

to be considered, and medical status/healing abilities become a greater concern. With larger surgical exposure, there is a potential for greater post-operative morbidity and complications. Sutures in a fully reflected buccal and/or palatal flaps become very critical for maintaining graft and tissue margins. Larger flaps and the likely increased swelling create an environment for potential early loss of sutures, surgical wound opening, loss of graft, and infections. Additionally, the combination of the technique sensitive nature of the procedure along with the large access flap result in significantly increased surgical treatment times. To create a near full arch flap on the facial and /or palatal, care must be taken to not excise important anatomy and prevent damage to marginal and papillary tissues. Once access is obtained, creating corticotomies would likely take the least amount of time of the entire procedure. The subsequent grafting and releasing of tissues can continue to extend surgical time. These procedural steps can accumulate to a relatively long procedure. Time alone could significantly affect the acceptance of this treatment from both the patient and/or clinician. Increased time also can relate to increased cost of the procedure. The cost would also be potentiated by the amount of grafting. Hence, the time/complexity related cost increase may also become a deterring factor in patient acceptance for PAOO. This technique has scientific literature suggesting its viability, but the patient/clinician acceptance needed to be addressed[1] (ref format). Recent techniques have been focusing on more minimally invasive approaches to accelerate tooth movement.

## Corticision

Park et al. (2006) was able to create cortical perforations using a modified scalpel (Park et al. 2006). This technique, known as corticision, did not require a flap. The scalpel could be placed interproximally and passed through the soft tissue all the way to the cortical bone. Then the scalpel could be hammered into the bone with a mallet to perforate the cortex. Lastly the

bone is luxated through the isolated vertical incision. The key point of this technique is that it can create the interproximal cortical perforations and induce the RAP effect without creating a full thickness flap. This was a major step in minimizing the amount of surgery to attempt to achieve the same type of accelerated tooth movement. These vertical incisions could also be left to heal by primary intention without significant time suturing. No papillary tissues are reflected, which helps prevent the risk of marginal and interdental tissue loss. Thus, the complexity, time, and cost can be reduced significantly while still providing accelerated tooth movement. One potential issue without a full thickness flap is the lack of access during creating the corticotomies. Without full access the prominence of the teeth may be less distinguishable and could result in accidental perforation into the tooth structure. Although there is limited evidence to suggest that perforating into cementum and dentin during a corticotomy results in pathological changes, perforating into the pulp can lead to possible devitalization, root resorption, and tooth loss. Thus, obtaining proper paralleled BWs, PAs, and ideally a CBCT are critical for analyzing the locations of corticotomies. Any sites radiographically determined to have roots in very close proximity should be avoided (Park et al. 2006). Additional precautions that can be taken when performing flapless corticotomies include palpation of root prominence and/or surgical guides derived from radiographs designed to avoid tooth structure. The interproximal crestal bone is another anatomical site that is at increased risk of damage without a fully reflected flap. Bone sounding in combination with radiographic examination should be used to avoid damage to crestal bone. Though there are risks involved with limited access, the benefits of the minimally invasive nature of a flapless corticotomy are profound. Less flapping means less surgical time and post-op morbidity. The cost could be reduced as well. Thus, this could increase case acceptance and clinician willingness to perform accelerated orthodontic treatment.

## Piezocision®: Minimally Invasive Accelerated Orthodontic Tooth Movement

In 2011, Dr. Sergai Dibart also developed a flapless technique for creating corticotomies, but with a piezosurgical instrument rather than a scalpel (Dibart et al. 2011). Dr. Tomaso Vercellotti developed the concept of utilizing the piezosurgical instrument to perform the corticotomies (Thomas et al. 2017). Surgical burs were traditionally used to perform corticotomies. Piezosurgical instruments provide certain advantages that traditional rotary instruments do not. Piezosurgical tips are designed to cut hard tissue without damaging soft tissue. In areas with proximity to intraosseous nerves, vessels, sinus membranes, and flapped tissue, having an instrument friendly to soft tissue reduces risk for iatrogenic injury. Also, piezosurgical instrument use has been suggested to result in beneficial osteogenic healing. Vercellotti et al. (2005) analyzed the histology of bone remodeling following bone removal from a carbide bur, diamond bur, and piezosurgical instrument. The study demonstrated that rotary instruments resulted in greater bone loss during remodeling then piezosurgical osteotomies (Vercellotti et al. 2005). Thus, the corticotomy induced by the piezosurgical instrument could prevent any advertent bone loss following the completion of tooth movement. It is because of these findings in conjunction with the safety of not damaging soft tissue that has coined this as a "friendly" alternative to utilizing a rotary bur. The full nature of the osteogenic effects of the piezosurgical units has not yet been identified. Histologic studies have suggested both catabolic and anabolic benefits (Baloul et al. 2011, Dibart et al. 2013, Vercellotti et al. 2005). Thus, it is conceivable to suggest that utilizing these alternative cutting instruments can either relatively increase or decrease the rate of tooth movement. If the cellular orchestration is simply modified to modulate pathogenic levels of inflammatory cytokines and cellular activity, then the RAP effect would simply be modulated by the piezoelectric stimulation of bone. This would allow safe continual bone turnover to facilitate faster tooth movement. If the potentiation of the piezo unit

results in an upregulation of osteoblastic and anabolic environment, then the length of RAP effect would be less. Bone would be rebuilding fast to respond to the injury resulting in a quicker response and decreased amount of time in which the bone is softer. This would result in a relative reduction in the acceleration of tooth movement. Histologic evidence regarding accelerated orthodontics and suggests a more catabolic upregulation and, thus, may help create less mineralized bone for the tooth to move through (Baloul et al. 2011, Dibart et al. 2013).

Dr. Dibart's technique, coined Piezocision, is a corticotomy technique utilizing isolated vertical incisions interproximally (Dibart et al. 2011). The incisions are similar to the corticision technique. They are made full thickness and do not include the crestal bone and papilla, and do not extend too far apically. The location of the vertical incisions are limited to just access bone in the areas required to create corticotomies. A rotary bur would most definitely harm the soft tissue if passed through this limited access. Using the piezosurgical tips, allows for safe cutting of hard tissue without damaging the soft tissue. The instrument is moved apico-coronally through the incision until the cortical bone has been perforated. The depth can be pre-determined by CBCT analysis or can be felt through the tactile difference of cortical vs. cancellous bone. Digitally based guides can also be fabricated based off radiographic and surface scan data. It is estimated that a minimum of 3-5mm of insertion of the piezoelectric tip through the soft tissue would be required to reach through the cortical bone. This depth can vary depending on the individual location of the corticotomy in the arch and bone thickness phenotype of an individual. As with PAOO, at least 2mm of crestal bone needs to be intact to preserve vitality of interproximal one height. Damaging this region may result in loss of interdental papilla height. With limited access, similar precautions as in corticision must be applied. Based from a combination of histomorphometric analysis and clinical observations, it is suggested that the span of the RAP effect is 1.5 teeth from site of the corticotomy (Dibart et al. 2013). This would allow the clinician to skip an interproximal site and

still obtain the RAP effect on the skipped area. The amount of controlled injury to induce the RAP effect is still to be determined in literature. The original concept of creating large mobile blocks of bone, which essentially could be considered distraction osteogenesis, could be considered a very large injury. As techniques progressed, the amount of injury became smaller. PAOO would only place corticotomies on the buccal surrounding the tooth and only disrupting the cortical bone. The PAOO technique has evolved to only need interproximal decortication. With the reduction in corticotomy injury size, similar RAP related tooth movement has been able to be accomplished (Makki et al. 2014).

Early rodent models by Dibart et al. (2013) and Baloul et al. (2011) were able to demonstrate a distinct and sufficient RAP effect histologically, which resulted in nearly twice the amount of tooth movement in the same span of time (Baloul et al. 2011, Dibart et al. 2013). The animal-based RAP effect was determined based off histological mineralized bone content in conjunction with osteoclastic activity and was determined to peak at approximately 28 days and return to baseline by 56 days. Similar findings by Baloul et al. (2011) was found but able to find fluctuations in cytokine markers suggesting the increase in bone turnover at approximately 3-5 weeks (Baloul et al. 2011). Based on clinical case series by Dibart et al. (2013), the amount of time the RAP effect is active in humans is like that found with PAOO techniques. It begins at approximately 1-2 weeks, peaks at 2 months, and returns to baseline at approximately 4 months. A recent randomized clinical trial compared conventional orthodontics with Piezocision and was able to demonstrate a nearly half reduction in treatment time (Charavet et al. 2016). Piezocision also allows for grafting to alter the tissue biotype. If grafting is desired, a tunnel can be used via the vertical incisions and bone or soft tissue grafts can be applied through the same incision. In grafting scenarios, sutures would be required to contain graft. The ability to graft can be a vital component of providing ideal pre-orthodontic periodontal care. A distinct advantage to

performing PAOO is that access is gained to graft and help maintain facial tissue stability. With the advancement of tunneling techniques, grafting can still be performed.

### Propel®

Another minimally invasive technique to decorticate uses the Propel® device (Alikhani et al. 2013). The Propel device is a threaded point with a depth stopper that is advanced through soft tissue and through the cortex creating micro-osteoperforations. Several punctures can be made at each interproximal site. These micro-osteoperforations have recently been shown in a rodent model to result in similar bone alterations as seen in other corticotomy techniques (Alikhani et al. 2015). A Clinical study has shown a near 50% reduction of treatment time as compared to conventional orthodontics. Once again, access is limited to observe osseous anatomy. Thus, similar precautions must be taken to prevent damage to dental structures, crestal bone, vital vessels/nerves, and marginal soft tissue. Due to the small size of the cortical perforation, it is recommended to perform 2-3 or more interproximally in sites desired to accelerate the movement. Micro-osteoperforations results in the least amount of access for grafting. There are no incisions in this technique. The tissue in the site of perforation is simply punctured. Large graft tissue cannot be performed in conjunction with micro-osteoperforations unless further access is made. It is recommended that micro-osteoperforations can be performed every 2 months when attempting to accelerate the movement of teeth. This is a relatively sooner re-entry rate for attempting to re-injure the bone. Thus, it may be in anticipation of a relatively shorter RAP effect due to the minimal nature of the cortical perforations.

### Pulsed Electromagnetic Field Therapy

More techniques are available for accelerated orthodontics that do not utilize decortication, such as pulsed electromagnetic fields (PEMF). Pulsed electromagnetic frequencies

utilize electromagnetic energy to elucidate an osteogenic response (Dogru et al. 2014, Esther 2010, Hannemann et al. 2014 ,Shupak et al. 2003, Sheneva et al. 2014, Stark and Sinclair 1987,). This transfer of energy may result in alterations of bone turnover and facilitate tooth movement (Dogru et al. 2014). Electromagnetic energy dictates atoms, molecules, cells, and organs. Essentially it is everywhere and is innately a major component of how nearly everything functions. The oral cavity has been shown to have an electromagnetic energy profile, and this value changes depending on the amount of restorations present (Skomro et al. 2012). Electromagnetic energy is a wave form of energy measured in units of wavelength. The most common form of electromagnetic radiation that is studied for medical/dental purposes is that within the visible light spectrum. Visible light wavelengths, such as those emitted by laser emitting devices, have also been indicated in having healing potential. Low intensity laser therapy (LILT) is a form of electromagnetic radiation to induce a healing response (Sheneva et al. 2014). LILT has been attempted for accelerated orthodontics but has shown limited response. Electromagnetic energy outside of the visible light spectrum is also utilized in both fields. A great example is radiographic imaging. X-rays fall on a shorter wavelength then visible light. Although it has been determined that osteogenic cells respond differently to various levels of electromagnetic radiation, the exact nature of this relationship is not well understood. Due to this lack of dose/reaction understanding, clinical studies have yet to establish the proper form of application for this type of energy. PEMF is created by an alternating magnetic resonance that creates a flow of electromagnetic energy. In this fashion, the electromagnetic energy is traveling at low frequencies between 6 Hz to 500 Hz, the range in which most biological activity can be found (Shupak et al. 2003). The alterations of current will result in changes in the frequencies. The rate of frequency change and the frequency itself are major factors in deriving a desired effect from the PEMF. Clinical studies observing fracture healing in long compact bones have demonstrated significant accelerated healing

potentials (Diniz et al. 2002, Hannemann et al. 2014). An example of electromagnetic radiation's direct effect on cellular response is noted in cellular membrane response. As an electromagnetic field is pulsed around a cell, pore permeability can change allowing water and ions to flow more readily between the inside and outside of the cell. This could drastically change concentration gradients and significantly affect cellular activity. Also, surface receptors such as epidermal growth factor receptors, can fluctuate in numbers resulting in altered functions (Satake 1990). Osteoclastogenesis has been shown to be decreased by PEMF via reduction of the $Ca^{2+}$-calcineurin-nuclear factor of activated T-cells signaling pathway (Zhang et al. 2017). The most agreed upon effect has been suggested to be the upregulation of osteoblast functionality (Diniz et al. 2002, Shupak et al. 2003, Kim 1990). These potential osteogenic affects can play a vital role in the periodontium, especially the alveolar bone. Implant studies in rabbit models using PEMF have shown to upregulate genetic control of osteoblastic activity and increase early bone to implant contact (Bambini et al. 2017, Barak et al. 2016).

Tooth movement with PEMF has been studied in a few animal models. A rodent model in which the upper two central incisors were separated orthodontically, PEMF was shown to increase the amount of tooth movement significantly more than control within the same amount of time (Dogru et al. 2014). The caveat is that this study only was conducted for 8 days, which can only reflect early stage changes. Cellular effects of PEMF in the periodontium have also been suggested. Human PDL fibroblasts where shown to have changes in spreading and adherence upon application of a PEMF in an in-vitro model (Kim 1990).

Typically, electromagnetic radiation is fluctuating constantly around our bodies. Sources of electromagnetic radiation include everything from our TV's to the radio broadcasting towers. This creates a field of energy, also known as "electrosmog" that may not necessarily be the range in which our cells ideally respond. A pulsed electromagnetic field (PEMF) can create a "bubble" of

desired electromagnetic radiation, cancelling outside sources and providing a targeted range of energy. Studies have attempted to isolate a range of electromagnetic radiation that cellular activity would respond favorably to (Shupak et al. 2003). By pulsing this controlled electromagnetic field, this energy could theoretically allow for increased desired cellular activity.

As mentioned before, bone may have piezoelectric properties. When strain is applied to bone, energy is dissipated related to the strain. Vice versa is true as well. If an electromagnetic energy is applied to the bone, then strain may occur. It may be conceivable that the piezoelectric instrument applies a specific range of strain to the bone related to the piezoelectricity used to power the internal ceramic rings. Ceramic rings within the handpiece of a piezoelectric unit are piezoelectric as well. Applying a set voltage results in strain. Pulsing this energy results in the ceramic rings to expand and compress rapidly. This vibration mechanically vibrates the piezoelectric tip inside the handpiece. This vibrational energy, once applied to bone, may result in an indirect transfer of energy to the bone. This specific dose of energy can dissipate further from the actual site of contact between instrument and bone. Thus, it is possible for an alteration in neighboring cellular activity to this spread of energy (Vercellotti et al. 2005). This dose would be likely a dampened version of what was used to apply the strain on the ceramic rings in the piezoelectric handpiece. It has been suggested that the 30kHz frequency range output from current piezoelectric surgical units can potentially result in osteogenic responses (Dibart et al. 2013). This may suggest that beyond creating a controlled cortical injury, the piezosurgical units can release electromagnetic radiations resulting in osteogenic changes in a similar fashion as PEMF. Studying the effects of PEMF in accelerated orthodontics could help better understand the nature of electromagnetic energy and its role in bone healing around teeth. Additionally, studying PEMF directly against piezosurgical instruments may shed light on the similarities or differences between the electromagnetic effects on the periodontium.

No matter the technique, accelerated orthodontics is designed to alter the periodontium to facilitate faster tooth movement. These newer more minimally invasive techniques have been studied for their effectiveness with little focus on the detailed effects on the periodontium. Current research has focused on the bone density as well as the number of local osteoclasts. Other factors such as the migration/polarization of fibroblasts, nature of vessels, periodontal attachment fiber turnover remain to be histologically described with these techniques. It is important to track the actions of fibroblasts and their relation to the periodontal ligament. Knowing how they differ amongst techniques is important for understanding how the attachment apparatus re-matures. Any disruption in this process could result in iatrogenic attachment loss. Understanding the alterations in the alveolar bone and tooth attachment apparatus is key to both learning about the techniques' effectiveness, as well as its relative safety. With a thorough investigation on the histological nature of the periodontal response to these accelerated orthodontic techniques, potential risks and benefits could further be elucidated.

The scientific literature has yet to support that any technique outperforms the other. Previous studies focus on a single technique and lack in histological support for its function. Therefore, the aim of this study is to compare the effects of several minimally invasive techniques on orthodontic tooth movement. An animal model has proved an efficacious model in testing tooth movement and allows for histological evaluation. Considering these techniques can alter the nature of the periodontium, it is important to have a comparative histological understanding of these techniques.

**Purpose:** The purpose of this study is to compare the clinical, radiological and histological effects of several minimally invasive accelerated orthodontic techniques including Piezocision, Propel and PEMF on orthodontic tooth movement on a rat model. The clinical analysis will allow the study to elucidate the difference in resulting rate of tooth movement. Radiographic information can show tooth movement amounts and bone density differences. The goal for the histological analysis was to evaluate density differences of the bone and the relative effects on the periodontium.

## Materials and Methods

This study has been approved by the Institutional Animal Care and Use Committee of Nova Southeastern University. The animals were obtained and acclimatized in the animal care facility for at least one week. Rats were placed in pairs in cages at the animal care facility. Cages were supplied with rat chow and water ad libitum. A total of 61 young adult laboratory rats (Sprague–Dawley; 280–320g, 8-9 weeks old) were used. The rodent model has two large central incisors that have long roots traveling nearly the full extent of the cranium. Then there is a large edentulous space until the posterior 3 molars are found. The three molars are in direct contact with each other and relatively straight in alignment. The root surface area is significantly smaller on the molars as compared to the central incisors. Thus, the central incisors serve as great anchor for mesialization of the posterior molars. The first molar mesialization towards the central incisors has been utilized as an animal model for orthodontic movement in several similar studies (Baloul et al. 2011, Dibart et al. 2013). Many of the concepts for the animal model to be used in this study was derived from prior studies attempting to understand the effectiveness of decortication and other orthodontic interventions (Ibrahim et al. 2017). Following similar protocols would allow for possible comparative analysis in the form of a meta-analysis. Prior PEMF studies used the creation of a diastema on the upper central incisors (Dogru et al. 2014). The length of the roots on the upper incisors transverse on the dorsum posteriorly nearly the entire length of the cranium. Using this model would not allow for sufficient decortication along the length of the root.

The animals were divided into 4 groups at baseline as shown in Figure 1: 1) Control (tooth movement alone; n = 15)-, 2) Piezocision (n = 15), 3) Propel (n= 15), and 4) PEMF (n = 15). Three time-points of 7, 21, and 49 days were studied based on the established intervals in previous

studies (Baloul et al. 2011, Dibart et al. 2013). Prior studies have demonstrated that early signs of the RAP effect in rodents are not distinguishable until the first week. The following 1 month has peak in activity with a decrease towards baseline after 2 months. The animals were fed softened rat chow and water ad libitum and weighed weekly. The left side of each animal's maxilla served as the experimental side, while the right side did not receive any treatment to ensure that the

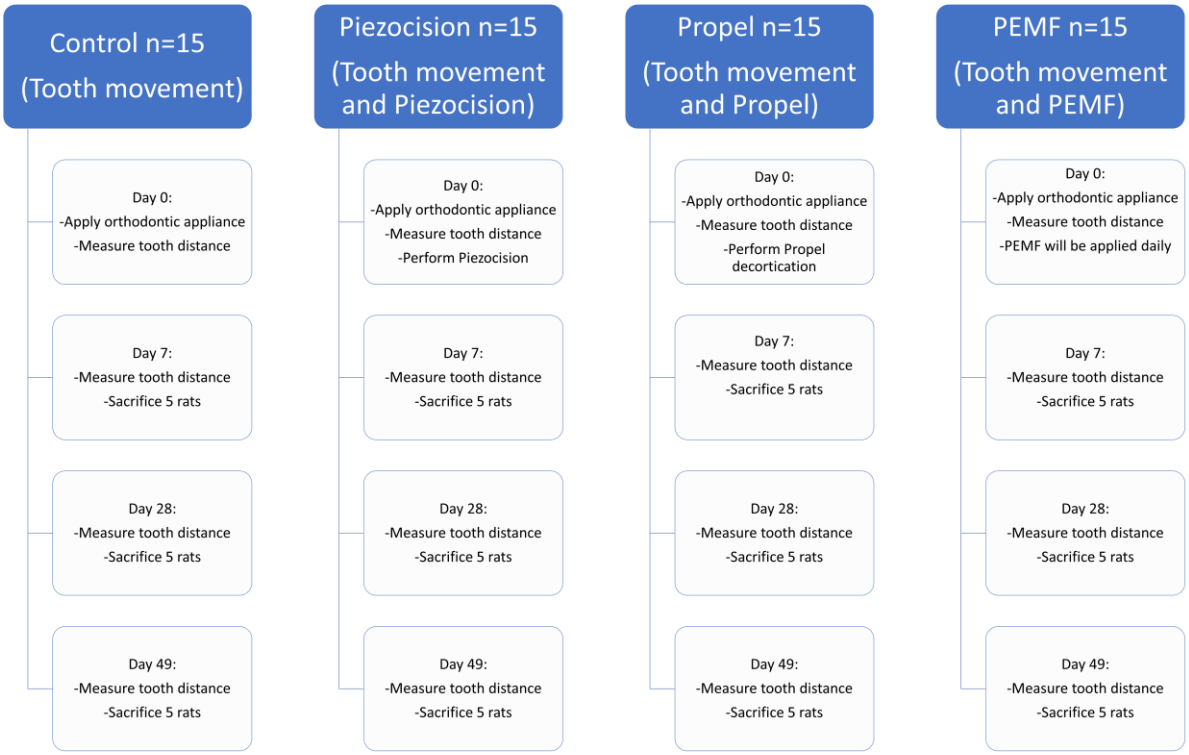


Figure 1: Summary of groups.

nourishment capacity of the animal was not impaired.

**Procedure**

All procedures were completed under general anesthesia with intraperitoneally administered ketamine (8mg/kg) and xylazine (5mg/kg) combination. Rats were initially weighed

with a scale at the animal facility immediately prior to transport. Rats were transferred in groups of 3-4 from animal care facility to operation site. Three empty recovery cages were brought as well. Rats were handled with care to expose abdomen to allow for intraperitoneal injection. Upon injection rats were replaced into recovery chamber until anesthesia could be visually determined. A stabilization operating table was created to minimize movement during procedure and facilitate access to the test molar. Once rats were anesthetized, a cephalometric X-Ray was taken using fixed markers on the stabilization table to hold the rats' heads and bodies in the same relative positions. The cephalometric X-Ray was utilized to detect any growth pattern anomalies between rats. To access the test molar, retraction was needed throughout the procedure. Two research assistants were required for retraction and rodent stabilization. To stabilize the rats head two fingers in a V-Shape was used to support the head with palm rest on the stabilization table. The rat's torso was stabilized with three fingers and palm rest on the stabilization table. Stabilization was required to prevent rat movement during the procedure. Careful pressure was used as to not inhibit the rats breathing. Visual signs of breathing and haptic feeling from the torso stabilizer was used to gauge the respiratory status of the rodent. Retraction was performed with a floss passed around lower and upper incisors separately. The floss was attached to cotton pliers which were extended out away from the rat's head to open the rat's mouth. Another finger from the torso stabilizer was sued to protrude the tongue. Managing the tongue allowed for better access and a patent airway. This access was used for all procedural steps required intra-oral access. A clinical measurement from the anterior extent of the incisors to the upper left first molar was taken with digital calipers. A digital caliper could not be used intraorally so a direct/indirect method was used to measure the space. A blunted wire was placed at the most distal extent of the gingival margin of the incisors and the most mesial extent of the gingival margin of the test molar. This distance was marked on the wire. The digital caliper was used to measure this difference as the baseline

clinical distance of the edentulous space. After measurements, rats were randomly assigned to one of the four groups. Then they were sub grouped to one of the three time points (7, 21, and 49 days).

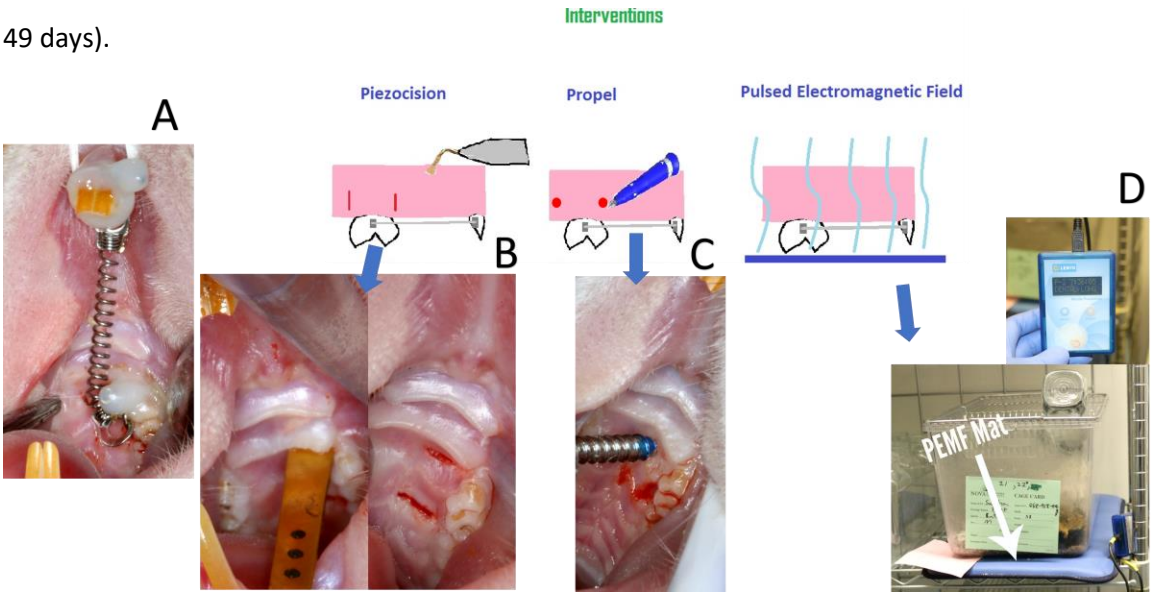


Figure 2: Interventions

All rats in this study received the same orthodontic appliance as depicted. A) Control group received only tooth movement with no other intervention. B) The Piezocision group received a corticotomy at the mesial and distal palatal of the test molar using a piezosurgical instrument. C) The Propel group received a decortication puncture using the device at the mesial and distal of the test molar. D) PEMF rats received a daily dose of approximately 7 hours set on a bone setting from a mat placed underneath the cage.

Piezocision and Propel groups required an intra-operative intervention that was performed after measurements. For the Piezocision group, an incision was done with a 15c blade on the mesial and distal of the first molar (Figure 2B). The decortication was performed with a piezoelectric surgical unit with a BS1 insert tip to a depth of 1 mm. The decortication was taken to the depth of 1 mm to assure that it has penetrated through the cortex and that depths were even on each rat. In addition to depth control, haptic feedback of the quality of bone was noted during the utilization of the piezosurgical depth to insure bone has been injured. The Piezoelectric unit is set to 30 kHz to allow for proper cutting efficiency without over heating the bone, and

potentially resulting in osteogenic effects via electromagnetic radiation. Corticotomy was performed with thorough irrigation. Suctioning and frequent breaks were taken to prevent aspiration of fluids in the airway. The incision created was minimal to only allow penetration of the BS1 tip. The incision was in position for primary closure and did not require suturing. Hemostasis was quickly acheived following pressure with a cotton tip.

For the Propel group, the device was modified to allow for application to a depth of 1mm at the distal and mesial of the test molar. The stopper was removed. A 1mm mark was made with permanent ink. The instrument tip was advanced with a twisting motion to penetrate soft tissue and decorticate (Figure 2C). Once the mark was reached the instrument was backed out. The penetration through the soft tissue places minimal trauma to soft tissues and does not require suturing. Typically, two to three penetrations are done on humans in clinical practice. Due to the size discrepancy of rat molars to human teeth, only one puncture was made at each site. Hemostasis was achieved using pressure with a cotton tip.

For pulsed electromagnetic field (PEMF, Lenyosys BioRegulation device), a device that is flat and has adjustable pulsing modes output was used. No extra intervention for this group was needed during the time the rat was under anesthesia. The device has several settings based on the desired effect. The device was set to a “dental” setting designed to emit frequencies that promote osteogenic properties. Plastic cages containing the rats were placed on top of the PEMF mats. The PEMF was applied daily for 7 hours during the duration of the project (Shupak et al. 2003). The mats PEMF range was tested utilizing a magnetic flux reader. The field created was measured to reach approximately 8-10 cm above the mat. This would allow for the rats to receive the full effect of the field while in the cage (Figure 2D). Two mats were used. If more than two cages were in the facility that were in the PEMF group, cages would be rotated to ensure all rats

PEMF group rats received the 7-hour dose daily. The mats were powered by AAA batteries and required a battery change after 2 uses. Batteries were changed after every 2nd cycle for each mat.

Placement of Orthodontic Appliances:

After interventions were performed, each rat had the orthodontic appliance placed (Figure 2). The orthodontic appliances chosen are of the smallest size and are applicable to rodent teeth. The tip of a stainless-steel ligature was slightly bent 2-4mm from the tip. A suture holder was used to pass the bent tip from the buccal to palatal on the interproximal of the first and second molar. If tissues were punctured, Cotton tip applicators were used to achieve hemostasis. After observing the tip enter the palatal area under the contact, the ligature was pulled and a 50g NiTi closed coiled spring was passed through the ligature to generate approximately 25-50g. This force is suggested to be a great amount but fall within the usual range used by other studies. Forces at 100g or greater tend to produce pathological conditions that can possibly inhibit tooth movement (Murphy et al. 2014, Ibrahim et al. 2017). A 50g force would allow for continuous application of forces 25g and greater for a longer duration (Figure 3). A 25g force application initially may begin to drop resulting in less force application as the study progresses. In conventional human orthodontics, forces are adjusted periodically to continue to achieve movement. Since forces are only being applied once at the beginning, the initial application of a higher force will allow for a better simulation of active forces throughout the entire duration of the study. The ligature was then twisted to tie off and place the spring at the mesial end of the test molar. The excess ligature was removed. The end of the tied ligature was covered in flowable composite and light cured. This would prevent the sharp edges of the ligature from harming the intra-oral environment. The size of the end of the ligature and composite was kept minimal to provide enough support without taking up significant room. The area was then sufficiently dried with cotton tips. Self-etch primer and bond was applied to the mesial of the test tooth and light

cured. Flowable composite was applied to the mesial aspect of the test tooth to cover both ligature and tooth. Composite was light cured. This was done to stabilize the ligature. Another ligature was passed through the other end of the NiTi closed coil spring. The ligature was continued to pass through the interproximal of the upper central incisors. The ligature was then tied off around both central incisors. This activated the closed coil spring between 25g to 50g (Figure 3). This range of force would allow for sufficient tooth movement with limited pathological tooth changes from excessive force issues. NiTi springs were tested with a force gauge to confirm that at the distance from first molar to central incisors produces a force of 25g to 50g. The central incisors were then dried thoroughly with a cotton tip. The self-etch primer and bond was applied to ligature and central incisors and light cured. Flowable composite was then applied to the tooth and ligature end. Composite was applied as to not cover incisal or occlusal surfaces. Composite was light cured. This device allows the upper left first molar to move mesially away from distal molars.


Figure 3: Force Gauge Measurements of the NiTi Spring used to apply orthodontic force.

After orthodontic appliance was placed, the rats were placed in the recovery cages. Rats were monitored for return to full consciousness. Upon full recovery rats were placed into new

cages. Rats were labelled based off number and not procedure. Only rats that were in the PEMF group were labeled on the cage as “PEMF” in addition to their number. All animals were permitted to move freely within their cages. All animals were housed in the same manner and provided softened rat chow and water ad libitum. The rat chow was soaked in distilled water for 2 hours and then placed in a feeding bowl in the cage. Rat chow had to be softened as hard pellets may dislodge the orthodontic appliance. Water and food was checked daily. Cages were cleaned, and rat bedding was changed weekly. Anterior orthodontic appliance was visible always. If any disturbance in the appliance was noted, rats were checked for stability of device. Rats would be once again sedated utilizing the same xylazine/ketamine mixture. The device would be checked. If device was deemed to be repairable, the device was re-adjusted. If device was unrepairable, a new device was placed. Rats were placed again in a recovery cage until they return to baseline level prior to sedation. If device was unrepairable within 24 hours prior to a designated sacrifice time, no repair was performed as rats would be sacrificed same day. Rats where weighed weekly and checked for general health. If any distinct activity suggesting systemic illness was noted rats would be further checked. Only one rat was noted to have a severe infection near the reproductive organs. Rat was noted to have severe weight loss, 1 week after intervention. Rat was euthanized at the one-week interval. One rat did not survive the intervention. This was the first rat, and retraction protocol was altered to include a forward retraction of the tongue.

The animals were sacrificed at 7, 21 and 49 days. Prior to euthanasia, rats where re-weighed. For euthanasia, an overdose of carbon dioxide and de-capitation was used. Rats were individually placed into a chamber with rubber hosing connected to a $CO_2$ tank. $CO_2$ flowed into chamber to euthanize rat. To confirm euthanasia, decapitation was performed after $CO_2$ application. After decapitation, the heads were placed in individual labeled jars of 10% formalin. Animal samples were given a new number by a separate research assistant. The new numbers

were correlated with the original number on a master data sheet, which would be used after data collection. This would then randomize the data collection as numbers on the cage would not indicate which treatment the rats had received. After one week in formalin, another cephalometric X-Ray was taken from the same fixed points to observe relative growth patterns of the rats. Also, clinical measurements were again taken from the incisors to the upper left first molar using a digital caliper.

**Histological Studies:**

After the euthanasia, the maxilla was removed with a diamond disc. Maxilla was placed into individual histology trays. Samples were prepared for histology inside of trays. Samples were fixed with 4 % paraformaldehyde, decalcified, embedded in paraffin, and transversely sectioned (5-8 µm thick) through the first, second, and third molar roots using a microtome producing five slide sections from apex to crown per arch. Slides at three different vertical levels of the roots (apical, middle, and coronal) were chosen and stained with hematoxylin and eosin. Histological analyses were performed for each sample.

In addition to observational data, histomorphometric analysis were performed utilizing ImageJ software. At the mid-root sectional slides, the area between the first molar roots including the roots (intra-radicular area) was used for histomorphometric analysis. The intra-radicular area was defined as a pentagon-shaped grid, each angle formed by the center of the five first molar

roots. Within the grids, the total amount of bone was recorded using ImageJ software to contrast the bone and was expressed as percentage of area with bone (Figure 4).

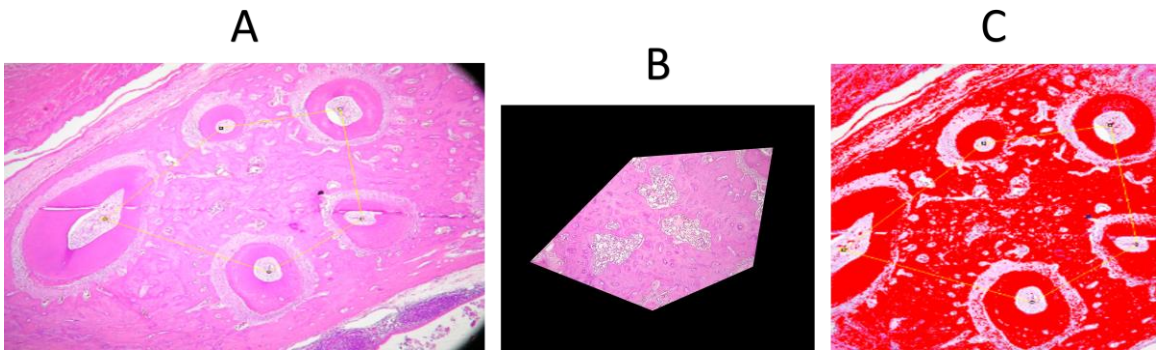


Figure 4. Histology Analysis

A) Midroot intra-radicular region of the test molar with the intra-radicular space outlined B) Cropped out grid of the intra-radicular space to be analyzed for bone density C) Bone is isolated utilizing ImageJ software to depict the relative density within the defined grid.

**Clinical Radiographic Measurements**

To determine the rate of tooth movement, a clinical and radiographic analysis were performed. For a clinical measurement, digital calipers were used to measure the amount of space between the first molar and the anterior teeth in the experimental quadrant at baseline and at time of sacrifice. Cone beam computed tomography (CBCT) of the rat jaws were taken and utilized for measurements. These measurements included changes in the distance from test first molar to second molar giving the distance the test tooth was orthodontically moved (Figure 5). Additionally, CBCT images were analyzed utilizing ImageJ software to determine relative grey intensity values around a fixed grid surrounding the test molars (Figure 5). The grid was designed to be 4.5x6.5mm with the test tooth centered in the grid at the mid-root level. The grid was placed axially on the tooth along the long axis of the tooth. The same grid was applied to the control side

as well. 4.5 x 6.5mm was chosen as it encompassed control molar and a sufficient space around that could demonstrate the potential limits of the RAP effect. A smaller field may have cut off the expansive demineralization created by accelerated orthodontics. Grey intensity values were derived from images using ImageJ software. The grey intensity values of the control side were compared to the test side. The samples were scanned after the maxilla was fixed in formalin for at least 24 hours. Timing and comparing to the control side were designed to reduce potential errors introduced by the CBCT scanner. The CBCT scanner was set to a child setting. These differences in grey intensity value correlate to varying levels of bone calcification or density around the test molar at time of sacrifice. Lastly, the CBCT images were used to determine the amount of migration the 3$^{rd}$ molar traveled despite not having any orthodontic forces. Anecdotal information regarding the rate and/or amount of movement of teeth that are not involved in the orthodontic movement has been regarded to be different in the presence of accelerated orthodontics. This measurement may elucidate if any differences in non-activated molar movement exists amongst the groups. Three separate bilateral anatomical points (posterior orbit, zygomatic arches, and the base of the incisors) were used to create a line of symmetry. The line of symmetry was placed on the distal aspect of the control side of third molar. The distance from this line to the test side of third molar was measured in millimeters.

Micro Computed Tomography (Micro CT) was performed to measure relative bone densities and extent of demineralization. Micro CT machine was acquired after the majority of the study had been complete. Not all samples were able to undergo Micro CT. Because of the limited

number of samples sent for Micro CT, it was not included in the results of this study.

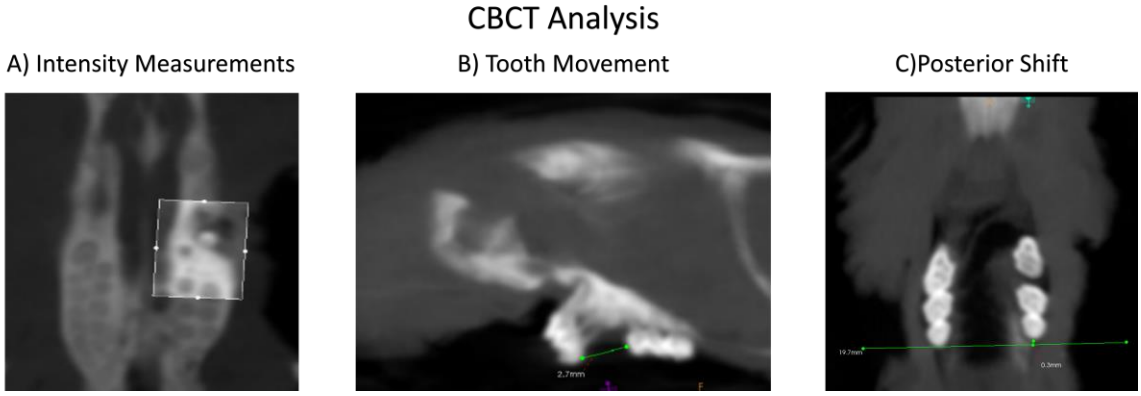


Figure 5: CBCT Analysis

A) Intensity measurements were made by creating a 4.5mm by 6.5mm grid with the midroot region of the test tooth centered in the grid. ImageJ software was used to obtain grey intensity values and compared to a grid placed on the contralateral side. This difference in intensity values created a relative decrease in intensity value representing a change in radiographic bone density. B) The distal contact point of the test molar to the mesial contact point of the second molar was used to measure the amount of tooth movement. All contacts were initially closed at baseline. Thus, any movement seen would represent movement due to orthodontic forces. C) Three consistent points were used to create a relative plane of symmetry to compare how much the 3$^{rd}$ molar had moved mesially on the test side as compared to the control side.

After de-randomization of data and further histological observations, it was determined that the orthodontic appliance may have not functioned properly on all samples. To reduce the variable of orthodontic appliance failure, samples deemed to not have any movement by day 21, less than 0.5mm of movement by day 49, and several appliance issues along the course of movement were omitted during a second round of statistical analysis. The 0.5mm mark was chosen because the average amount of movement in controls was over 1mm after first round of analysis. The samples with 0.5mm of movement correlated with appliance issues, such as broken anterior composite or dislodged posteriors ligature. The histology also resembled baseline normal

architecture as if orthodontic movement had ceased. The combination of these factors may suggest possible appliance issues and creates a potential uncontrolled variable.

**Statistical Analysis:**

To look for the change in clinical tooth distance (Incisal-Molar or IM distance); CBCT intensity values (CBCT Intensity Difference); CBCT based tooth distance from the second molar to first molar (CBCT tooth distance); amount of movement of the distal most molar mesially (Posterior Shift); and the histologic bone density (Histology Inter-Root Bone Percentage or Histology Bone Difference Percentage) we used a two-way ANOVA. The fixed effects were time (Day 7, Day 21, and day 49), group (control, PEMF, Propel, Piezocision), and the interaction of group by time. The co-variates were rat beginning and sacrifice weight. Post-hoc Tukey tests were also conducted. Statistical analysis was performed again after samples were omitted due to potential failures in the orthodontic appliances.

# Results

**Change in Incisor to Molar (IM) Distance**

For the change (baseline distance minus either 7,21, or 49 day time points) in the clinically measured distance of the central incisor to the test molar, we found a significant difference by time [$F$ (3, 42) = 36.52, $p < 0.001$, $\eta^2 = 54\%$], but no difference between groups ($p = 0.964$), or the interaction of group by time ($p = 0.715$). A post hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; no difference was found between day-7 and day-21 ($p = 0.440$) (Table 1).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 49 | 7 | 1.95 | 1.32 | 2.57 | <.0001* |
| 49 | 21 | 1.63 | 1.08 | 2.19 | <.0001* |
| 21 | 7 | 0.31 | -0.29 | 0.92 | 0.440 |

Table 1: Pairwise Comparisons of clinically measured distance of the central incisor to test molar. *Statistically significant difference (p< 0.05).

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I-M Distance Per (mm) | | | | I-M Distance Per (mm) | | | | I-M Distance Per (mm) | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 14.36 | 1.25 | 12.60 | 15.70 | 14.74 | 0.69 | 13.60 | 15.30 | 14.73 | 0.49 | 14.30 | 15.20 |
| PEMF | 14.92 | 0.54 | 14.10 | 15.60 | 14.34 | 0.50 | 13.70 | 15.10 | 14.44 | 0.13 | 14.30 | 14.60 |
| Propel | 14.68 | 0.33 | 14.20 | 15.10 | 14.62 | 0.33 | 14.20 | 15.00 | 14.46 | 0.09 | 14.30 | 14.50 |
| Piezocision | 14.92 | 0.20 | 14.60 | 15.10 | 14.67 | 0.33 | 14.30 | 15.10 | 14.52 | 0.44 | 13.90 | 14.90 |
| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
| | I-M Distance Post (mm) | | | | I-M Distance Post (mm) | | | | I-M Distance Post (mm) | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 13.72 | 1.07 | 12.10 | 14.90 | 13.62 | 0.77 | 12.40 | 14.20 | 11.90 | 1.05 | 10.90 | 13.00 |
| PEMF | 14.16 | 0.31 | 13.90 | 14.70 | 13.53 | 0.43 | 12.90 | 13.90 | 12.38 | 0.58 | 12.00 | 13.40 |
| Propel | 14.16 | 0.56 | 13.30 | 14.70 | 13.72 | 0.53 | 13.10 | 14.30 | 11.90 | 0.73 | 11.20 | 12.90 |
| Piezocision | 14.36 | 0.40 | 14.00 | 14.80 | 13.44 | 0.46 | 12.80 | 14.00 | 11.55 | 0.86 | 10.50 | 12.30 |
| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
| | I-M Distance Change (mm) | | | | I-M Distance Change (mm) | | | | I-M Distance Change (mm) | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 0.64 | 0.38 | 0.20 | 1.20 | 1.12 | 0.58 | 0.60 | 2.00 | 3.00 | 1.51 | 1.30 | 4.20 |
| PEMF | 0.76 | 0.59 | 0.10 | 1.50 | 0.80 | 0.77 | -0.20 | 1.40 | 2.06 | 0.55 | 1.10 | 2.50 |
| Propel | 0.52 | 0.39 | 0.10 | 0.90 | 0.90 | 0.63 | -0.10 | 1.50 | 2.56 | 0.69 | 1.60 | 3.20 |
| Piezocision | 0.56 | 0.38 | 0.10 | 1.00 | 1.14 | 0.51 | 0.50 | 1.80 | 2.90 | 0.81 | 1.90 | 3.70 |

Table 2: Descriptive Statistics for IM Distance

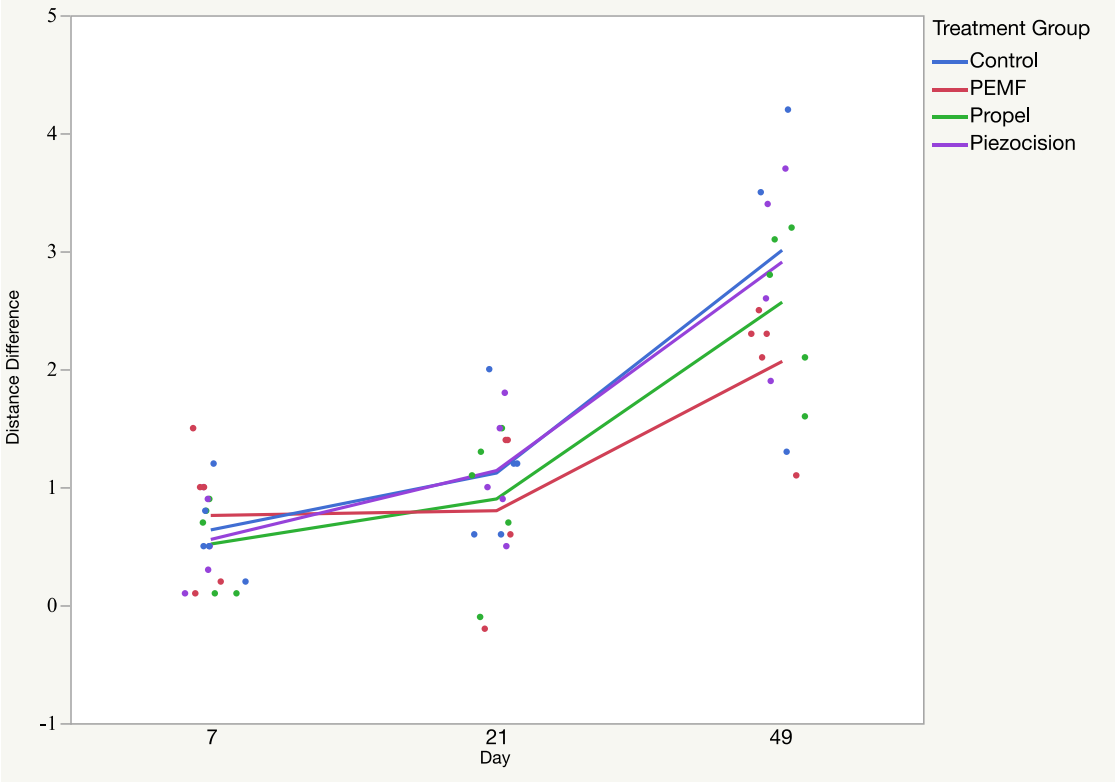


Figure 6. IM Distance measured by Distance Difference between initial measurement at baseline and at time of sacrifice.

**Change in CBCT Intensity**

For the difference in the CBCT intensity values around the test molar roots versus the contralateral first molar roots, we found a significant difference by time [$F$ (3, 39) = 14.21, $p$ < 0.001, $\eta^2$ = 28%], but no difference between groups ($p$ = 0.739, or the interaction of group by time ($p$ = 0.177). A post hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p$ < 0.05; no difference was found between day-7 and day-21 ($p$ = 0.440) (Table 3).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 49 | 7 | 29.70 | 5.80 | 15.57 | <.0001* |
| 49 | 21 | 20.42 | 5.10 | 8.00 | <.0001* |
| 21 | 7 | 9.29 | 5.29 | -3.59 | 0.197 |

Table 3: Pairwise Comparisons for CBCT Intensity Change
*Statistically significant difference (p< 0.05).

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Intensity Left | | | | CBCT Intensity Left | | | | CBCT Intensity Left | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 154.20 | 21.43 | 124.50 | 172.80 | 150.40 | 18.02 | 122.70 | 167.50 | 144.15 | 12.03 | 128.30 | 153.90 |
| PEMF | 164.58 | 8.71 | 155.40 | 173.30 | 149.90 | 15.74 | 131.20 | 161.50 | 146.73 | 16.81 | 124.80 | 160.30 |
| Propel | 149.85 | 27.59 | 112.00 | 178.10 | 154.76 | 9.36 | 144.10 | 166.20 | 138.40 | 15.34 | 122.10 | 163.50 |
| Piezocision | 144.60 | 25.45 | 116.60 | 172.20 | 131.55 | 20.87 | 101.40 | 147.60 | 116.10 | 30.69 | 80.80 | 136.40 |

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Intensity Right | | | | CBCT Intensity Right | | | | CBCT Intensity Right | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 163.28 | 29.98 | 120.70 | 186.90 | 156.68 | 18.40 | 129.20 | 178.10 | 170.80 | 22.04 | 138.50 | 184.90 |
| PEMF | 177.56 | 8.79 | 164.30 | 188.90 | 164.48 | 16.98 | 134.50 | 176.30 | 175.98 | 10.89 | 164.90 | 190.90 |
| Propel | 165.75 | 23.16 | 131.20 | 179.60 | 176.80 | 4.64 | 172.20 | 182.50 | 183.44 | 3.05 | 180.10 | 187.50 |
| Piezocision | 152.04 | 20.35 | 133.40 | 174.90 | 170.75 | 10.12 | 159.10 | 183.70 | 178.57 | 1.93 | 177.40 | 180.80 |

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Intensity Change | | | | CBCT Intensity Change | | | | CBCT Intensity Change | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 9.08 | 8.74 | -3.80 | 14.90 | 6.28 | 6.95 | -5.80 | 10.60 | 26.65 | 11.02 | 10.20 | 33.60 |
| PEMF | 12.98 | 6.26 | 4.90 | 21.10 | 14.58 | 12.11 | 3.30 | 34.90 | 29.25 | 24.92 | 12.50 | 66.10 |
| Propel | 15.90 | 10.16 | 1.50 | 25.30 | 22.04 | 10.70 | 9.40 | 36.70 | 45.04 | 15.39 | 22.20 | 65.40 |
| Piezocision | 7.44 | 6.86 | 0.40 | 16.80 | 39.20 | 25.80 | 11.50 | 69.90 | 62.47 | 29.66 | 44.40 | 96.70 |

Table 4: Descriptive Statistics for CBCT Intensity

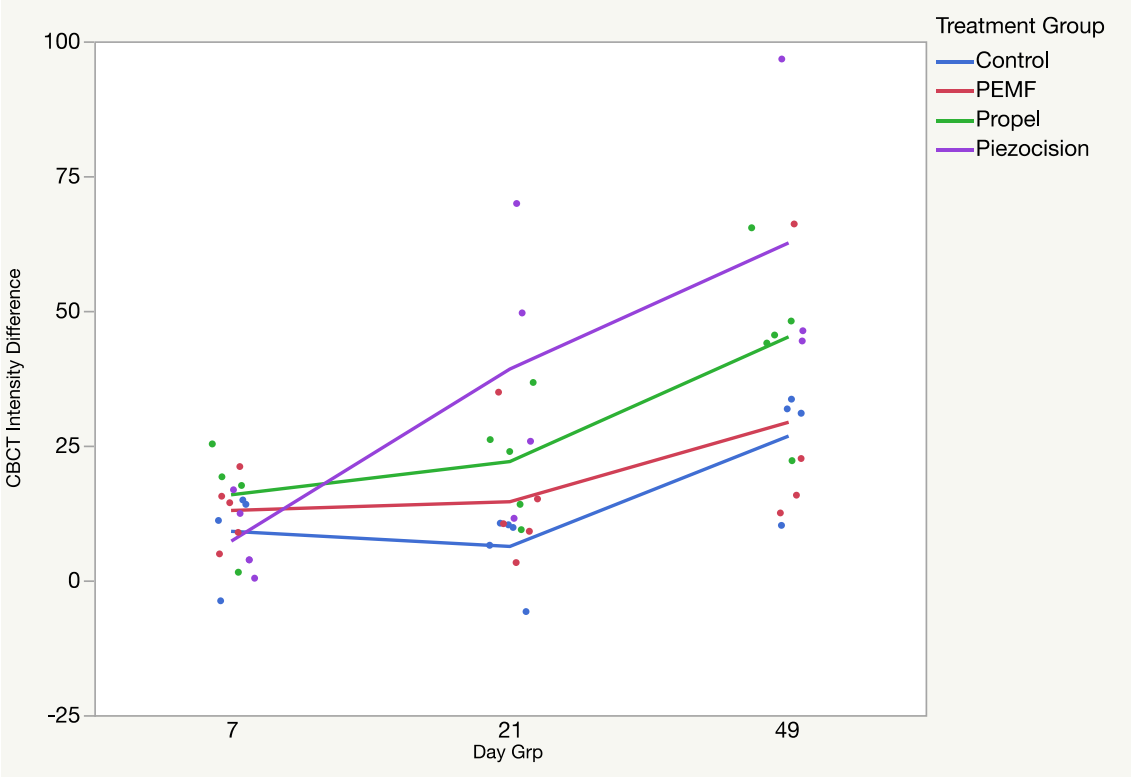


Figure 7: CBCT Intensity Difference

**Change in CBCT Tooth Distance**

For the CBCT measured difference of the test molar to the second molar, we found a significant difference by time [$F$ (3, 41) = 36.35, $p < 0.001$, $\eta^2 = 55\%$], but no difference between groups ($p = 0.997$), or the interaction of group by time ($p = 0.553$). A post hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; no difference was found between day-7 and day-21 ($p = 0.440$) (Table 5).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 49 | 7 | 2.03 | 0.27 | 1.36 | <.0001* |
| 49 | 21 | 1.52 | 0.27 | 0.86 | <.0001* |
| 21 | 7 | 0.51 | 0.26 | -0.13 | 0.142 |

Table 5: Pairwise Comparisons for CBCT Tooth Distance
*Statistically significant difference (p< 0.05).

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Tooth Distance | | | | CBCT Tooth Distance | | | | CBCT Tooth Distance | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 0.15 | 0.19 | 0.00 | 0.40 | 0.42 | 0.46 | 0.00 | 1.10 | 2.13 | 1.51 | 0.50 | 3.60 |
| PEMF | 0.18 | 0.16 | 0.00 | 0.40 | 0.58 | 0.70 | 0.00 | 1.70 | 1.35 | 1.07 | 0.40 | 2.70 |
| Propel | 0.10 | 0.08 | 0.00 | 0.20 | 1.04 | 1.11 | 0.00 | 2.30 | 2.24 | 1.28 | 0.60 | 4.00 |
| Piezocision | 0.04 | 0.05 | 0.00 | 0.10 | 0.45 | 0.34 | 0.00 | 0.80 | 2.87 | 0.50 | 2.40 | 3.40 |

Table 6: Descriptive Statistics for CBCT Tooth Distance

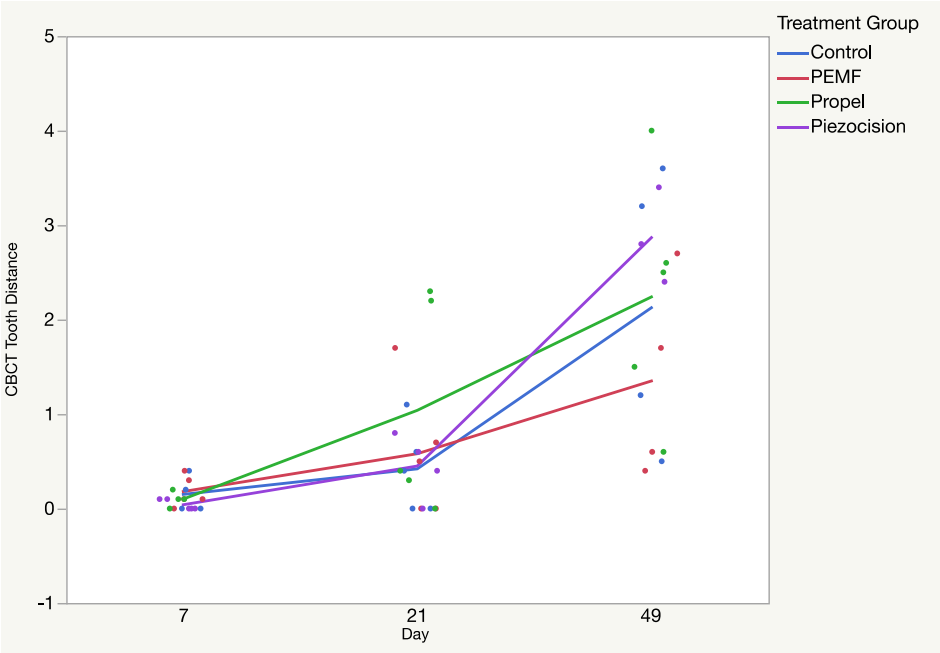


Figure 8: CBCT Tooth Distance

**Change in Posterior Shift**

To look for the change in CBCT tooth posterior shift we used a two-way ANOVA. The fixed effects were time (Day 7, Day 21, and day 49), group (control, PEMF, Propel, Piezocision), and the interaction of group by time. The co-variates were rat beginning and sacrifice weight. We found a significant difference by time [$F$ (3, 44) = 70.50, $p < 0.001$, $\eta^2 = 55\%$], but no difference between

groups ($p$ = 0.426), or the interaction of group by time ($p$ = 0.116). A post-hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; day-21 was significantly different from day-7 at $p < 0.05$ (Table 7).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 49 | 7 | 0.62 | 0.05 | 0.49 | <.0001* |
| 49 | 21 | 0.40 | 0.05 | 0.29 | <.0001* |
| 21 | 7 | 0.22 | 0.05 | 0.10 | <.0001* |

Table 7: Pairwise Comparisons for Posterior Shift
*Statistically significant difference (p< 0.05).

| | Day 7 CBCT Posterior Shift | | | | Day 21 CBCT Posterior Shift | | | | Day 49 CBCT Posterior Shift | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 0.05 | 0.10 | 0.00 | 0.20 | 0.24 | 0.13 | 0.00 | 0.30 | 0.55 | 0.06 | 0.50 | 0.60 |
| PEMF | 0.16 | 0.09 | 0.10 | 0.30 | 0.30 | 0.07 | 0.20 | 0.40 | 0.68 | 0.18 | 0.40 | 0.90 |
| Propel | 0.04 | 0.05 | 0.00 | 0.10 | 0.34 | 0.05 | 0.30 | 0.40 | 0.90 | 0.31 | 0.70 | 1.40 |
| Piezocision | 0.13 | 0.10 | 0.00 | 0.20 | 0.35 | 0.14 | 0.20 | 0.60 | 0.66 | 0.15 | 0.50 | 0.90 |

Table 8: Descriptive Statistics for Posterior Shift

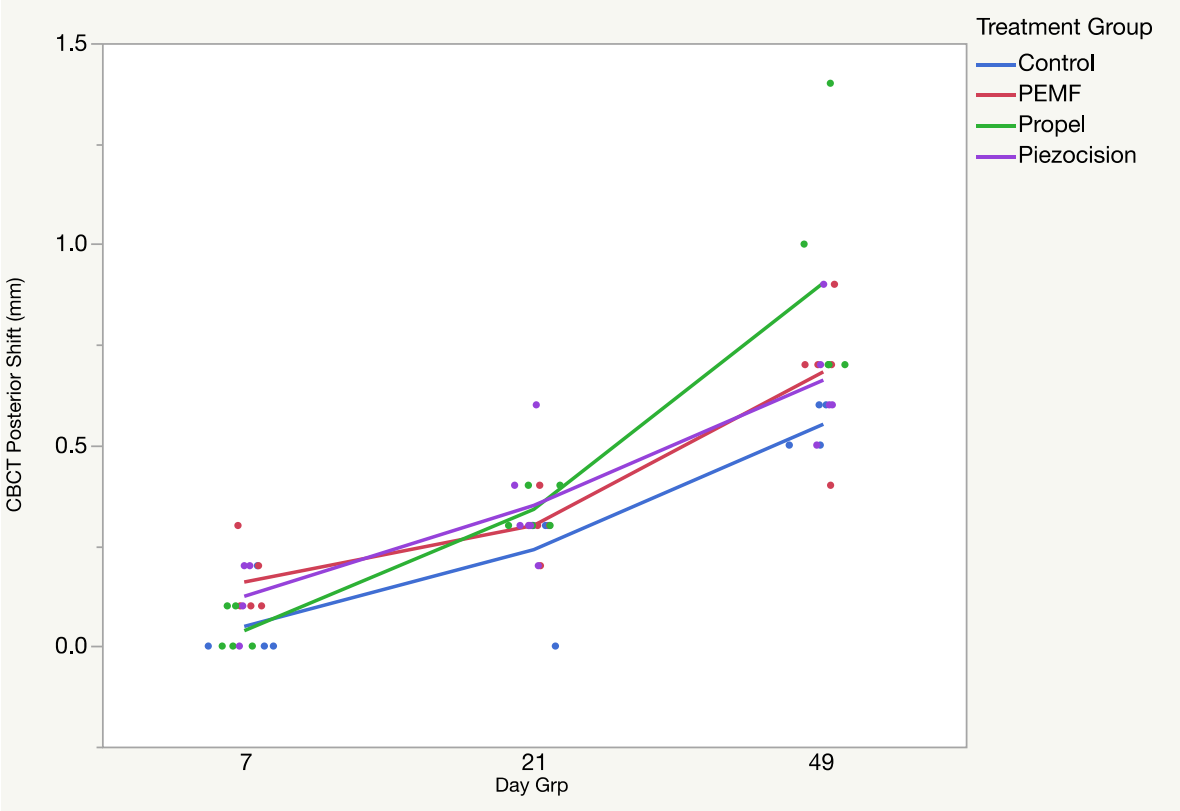


Figure 9: CBCT Posterior Shift

**Change in Histological Inter-Root Bone Percentage**

For the differences in the amount of measured histological bone density percentage around the intra-radicular midroot region of the test molar, we found no significant difference by time ($p$ = 0.554), and no difference between groups ($p$ = 0.946), or the interaction of group by time ($p$ = 0.697).

| | Day 7 Bone Percentage | | | | Day 21 Bone Percentage | | | | Day 49 Bone Percentage | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 58.93 | 8.27 | 49.30 | 69.30 | 70.38 | 5.87 | 63.80 | 79.70 | 75.05 | 6.59 | 67.80 | 83.40 |
| PEMF | 63.18 | 12.17 | 41.80 | 72.10 | 69.90 | 10.16 | 57.00 | 84.30 | 73.64 | 8.87 | 60.00 | 81.40 |
| Propel | 59.38 | 14.51 | 40.30 | 75.50 | 59.58 | 17.88 | 30.00 | 76.50 | 65.80 | 11.60 | 47.60 | 79.60 |
| Piezocision | 60.06 | 8.32 | 46.10 | 66.90 | 60.50 | 12.25 | 45.30 | 75.90 | 59.94 | 15.33 | 44.30 | 81.40 |
| | Day 7 Bone Percentage Control | | | | Day 21 Bone Percentage Control | | | | Day 49 Bone Percentage Control | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 79.20 | 1.64 | 77.30 | 81.30 | 77.32 | 9.11 | 64.70 | 87.70 | 79.78 | 7.59 | 68.90 | 86.10 |
| PEMF | 77.96 | 2.45 | 74.60 | 80.70 | 78.12 | 9.69 | 63.80 | 88.10 | 84.06 | 5.11 | 77.50 | 90.70 |
| Propel | 74.28 | 7.44 | 66.80 | 84.30 | 75.94 | 3.99 | 71.80 | 81.40 | 79.36 | 6.82 | 69.30 | 87.20 |
| Piezocision | 76.86 | 4.20 | 70.30 | 81.00 | 79.18 | 5.41 | 70.00 | 84.80 | 80.00 | 5.88 | 70.50 | 85.00 |
| | Day 7 Bone Percentage Change | | | | Day 21 Bone Percentage Change | | | | Day 49 Bone Percentage Change | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 20.28 | 6.63 | 12.10 | 28.00 | 6.92 | 6.38 | 0.90 | 17.10 | 4.70 | 7.82 | -3.70 | 12.80 |
| PEMF | 14.80 | 12.99 | 6.90 | 37.90 | 8.22 | 6.19 | 2.90 | 18.40 | 10.40 | 12.42 | -0.50 | 30.70 |
| Propel | 14.88 | 13.56 | 0.40 | 29.00 | 16.36 | 15.85 | 2.10 | 41.80 | 13.58 | 9.08 | 1.60 | 21.70 |
| Piezocision | 16.84 | 5.63 | 10.00 | 24.20 | 18.72 | 12.84 | 9.00 | 37.70 | 20.04 | 14.98 | -0.90 | 38.40 |

Table 9: Descriptive Statistics for Histological Inter-Root Bone Percentage

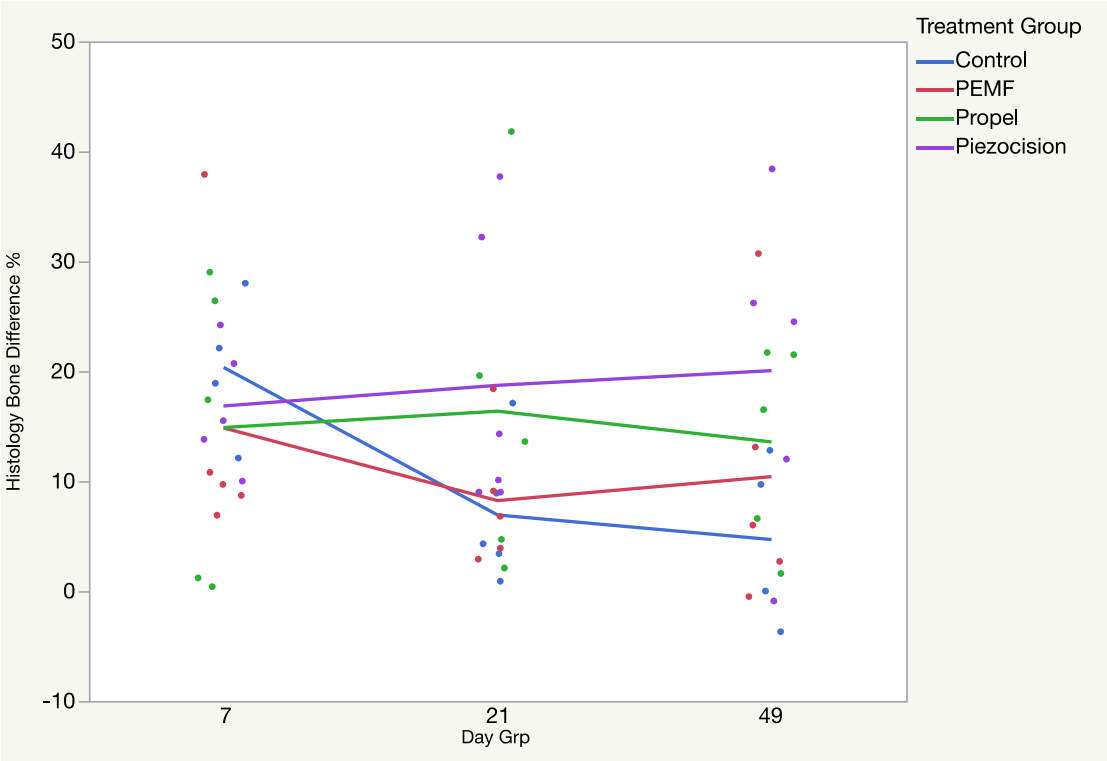


Figure 10: Histological Bone Density Difference Percentage in the Intra-Radicular

After removal of the samples that were considered to have failed orthodontic appliance. The same statistical analysis was performed.

**Change in Incisor to Molar Distance**

We found a significant difference by time [$F$ (2, 29) = 14.17, $p < 0.001$, $\eta^2 = 64\%$], but no difference between groups ($p = 0.874$), or the interaction of group by time ($p = 0.853$). A post hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; no difference was found between day-7 and day-21 ($p = 0.089$) (Table 10).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 21 | 7 | 0.60 | -0.08 | 1.28 | 0.089 |
| 49 | 7 | 1.99 | 1.34 | 2.64 | p < 0.001* |
| 49 | 21 | 1.39 | 0.70 | 2.08 | p < 0.001* |

Table 10: Pairwise Comparisons for IM Distance (samples removed)

*Statistically significant difference (p< 0.05).

| | Day 7 I-M Distance Per (mm) | | | | Day 21 I-M Distance Per (mm) | | | | Day 49 I-M Distance Per (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 14.50 | 0.82 | 13.80 | 15.40 | 14.93 | 0.32 | 14.70 | 15.30 | 14.73 | 0.49 | 14.30 | 15.20 |
| PEMF | 14.92 | 0.54 | 14.10 | 15.60 | 14.60 | 0.44 | 14.30 | 15.10 | 14.37 | 0.12 | 14.30 | 14.50 |
| Propel | 14.67 | 0.45 | 14.20 | 15.10 | 14.67 | 0.31 | 14.40 | 15.00 | 14.46 | 0.09 | 14.30 | 14.50 |
| Piezocision | 14.93 | 0.24 | 14.60 | 15.10 | 14.55 | 0.24 | 14.30 | 14.80 | 14.45 | 0.48 | 13.90 | 14.90 |
| | Day 7 I-M Distance Post (mm) | | | | Day 21 I-M Distance Post (mm) | | | | Day 49 I-M Distance Post (mm) | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 13.87 | 0.49 | 13.30 | 14.20 | 13.87 | 0.49 | 13.30 | 14.20 | 11.88 | 1.06 | 10.90 | 13.00 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PEMF | 14.16 | 0.31 | 13.90 | 14.70 | 13.30 | 0.57 | 12.90 | 13.70 | 12.08 | 0.11 | 12.00 | 12.20 |
| Propel | 13.87 | 0.55 | 13.30 | 14.40 | 13.37 | 0.31 | 13.10 | 13.70 | 11.91 | 0.73 | 11.20 | 12.90 |
| Piezocision | 14.25 | 0.37 | 14.00 | 14.80 | 13.04 | 0.68 | 12.20 | 13.70 | 11.33 | 0.91 | 10.50 | 12.30 |

| | Day 7 I-M Distance Change (mm) | | | | Day 21 I-M Distance Change (mm) | | | | Day 49 I-M Distance Change (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 0.63 | 0.51 | 0.20 | 1.20 | 1.07 | 0.81 | 0.60 | 2.00 | 2.98 | 1.51 | 1.30 | 4.20 |
| PEMF | 0.76 | 0.59 | 0.10 | 1.50 | 1.40 | 0.00 | 1.40 | 1.40 | 2.29 | 0.20 | 2.10 | 2.50 |
| Propel | 0.80 | 0.10 | 0.70 | 0.90 | 1.30 | 0.20 | 1.10 | 1.50 | 2.55 | 0.68 | 1.60 | 3.20 |
| Piezocision | 0.68 | 0.33 | 0.30 | 1.00 | 1.51 | 0.77 | 0.94 | 2.60 | 3.00 | 0.96 | 1.90 | 3.70 |

Table 11: Descriptive Statistics for IM Distance (samples removed)

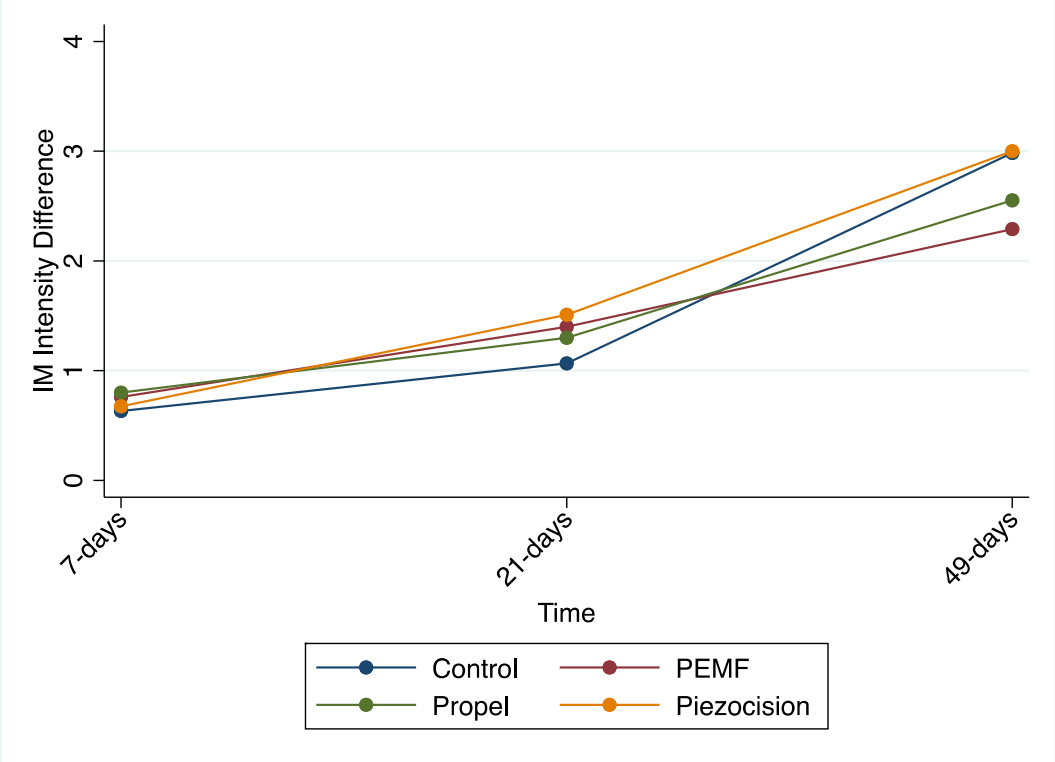


Figure 11: IM Distance (samples removed)

**Change in CBCT Intensity**

We found a significant difference by time [$F$ (2, 29) = 13.77, $p < 0.001$, $\eta^2 = 28\%$], between Piezocision and Control group [$F$ (3, 29) = 6.33, $p = 0.002$, $\eta^2 = 28\%$ , and also with Piezocision and PEMF $p = 0.012$. No difference was found for the interaction of group by time ($p = 0.059$) (Table 13) in bone density measurements in CBCT images measured by grey value intensity. A post-hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; no difference was found between day-7 and day-21 ($p = 0.440$) (Table 12).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 21 | 7 | 14.67 | 0.00 | 29.34 | 0.050* |
| 49 | 7 | 28.80 | 15.24 | 42.35 | p < 0.001* |
| 49 | 21 | 14.13 | -0.55 | 28.80 | 0.061 |

Table 12: Pairwise Comparisons of CBCT Intensity Change by time (samples removed)
*Statistically significant difference (p< 0.05).

| Group | Group | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| PEMF | Control | 5.64 | -12.17 | 23.46 | 0.824 |
| Propel | Control | 13.94 | -3.88 | 31.76 | 0.167 |
| Piezocision | Control | 28.43 | 9.56 | 47.30 | 0.002* |
| Propel | PEMF | 8.30 | -9.27 | 25.86 | 0.578 |
| Piezocision | PEMF | 22.78 | 4.15 | 41.41 | 0.012 * |
| Piezocision | PEMF | 14.49 | -4.14 | 33.12 | 0.171 |

Table 13: Pairwise Comparisons of CBCT Intensity Change by Groups (samples removed)
*Statistically significant difference (p< 0.05).

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Intensity Left | | | | I-M Distance Left | | | | I-M Distance Left | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 154.57 | 26.23 | 124.5 | 172.8 | 156.00 | 12.62 | 142.50 | 167.50 | 144.15 | 12.03 | 128.30 | 153.90 |
| PEMF | 164.58 | 8.71 | 155.4 | 173.3 | 142.20 | 16.52 | 131.20 | 161.20 | 148.20 | 20.27 | 124.80 | 160.30 |
| Propel | 140.43 | 24.69 | 112 | 156.4 | 154.50 | 7.28 | 146.10 | 158.80 | 138.40 | 15.34 | 122.10 | 163.50 |
| Piezocision | 137.98 | 23.9 | 116.6 | 172.2 | 117.75 | 23.12 | 101.40 | 134.10 | 116.10 | 30.69 | 80.80 | 136.40 |

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Intensity Right | | | | CBCT Intensity Right | | | | CBCT Intensity Right | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 162.97 | 36.71 | 120.70 | 186.90 | 166.23 | 12.72 | 152.80 | 178.10 | 170.80 | 22.04 | 138.50 | 184.90 |
| PEMF | 177.56 | 8.79 | 164.30 | 188.90 | 159.97 | 22.35 | 134.50 | 176.30 | 179.67 | 9.81 | 172.80 | 190.90 |
| Propel | 161.13 | 26.01 | 131.20 | 178.20 | 175.87 | 5.76 | 172.20 | 182.50 | 183.44 | 3.05 | 180.10 | 187.50 |
| Piezocision | 146.33 | 18.29 | 133.40 | 172.60 | 177.50 | 8.77 | 171.30 | 183.70 | 178.57 | 1.93 | 177.40 | 180.80 |

| | Day 7 | | | | Day 21 | | | | Day 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBCT Intensity Change | | | | CBCT Intensity Change | | | | CBCT Intensity Change | | | |
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 8.40 | 10.57 | 3.80 | 14.90 | 10.23 | 0.40 | 9.80 | 10.60 | 26.65 | 11.02 | 10.20 | 33.60 |
| PEMF | 12.98 | 6.26 | 4.90 | 21.10 | 17.77 | 15.97 | 3.30 | 34.90 | 31.47 | 30.04 | 12.50 | 66.10 |
| Propel | 20.70 | 4.06 | 17.60 | 25.30 | 21.37 | 6.39 | 14.10 | 26.10 | 45.04 | 15.39 | 22.20 | 65.40 |
| Piezocision | 8.35 | 7.57 | 0.40 | 16.80 | 59.75 | 14.35 | 49.60 | 69.90 | 62.47 | 29.66 | 44.40 | 96.70 |

Table 14: Descriptive Statistics for CBCT Intensity (samples removed)

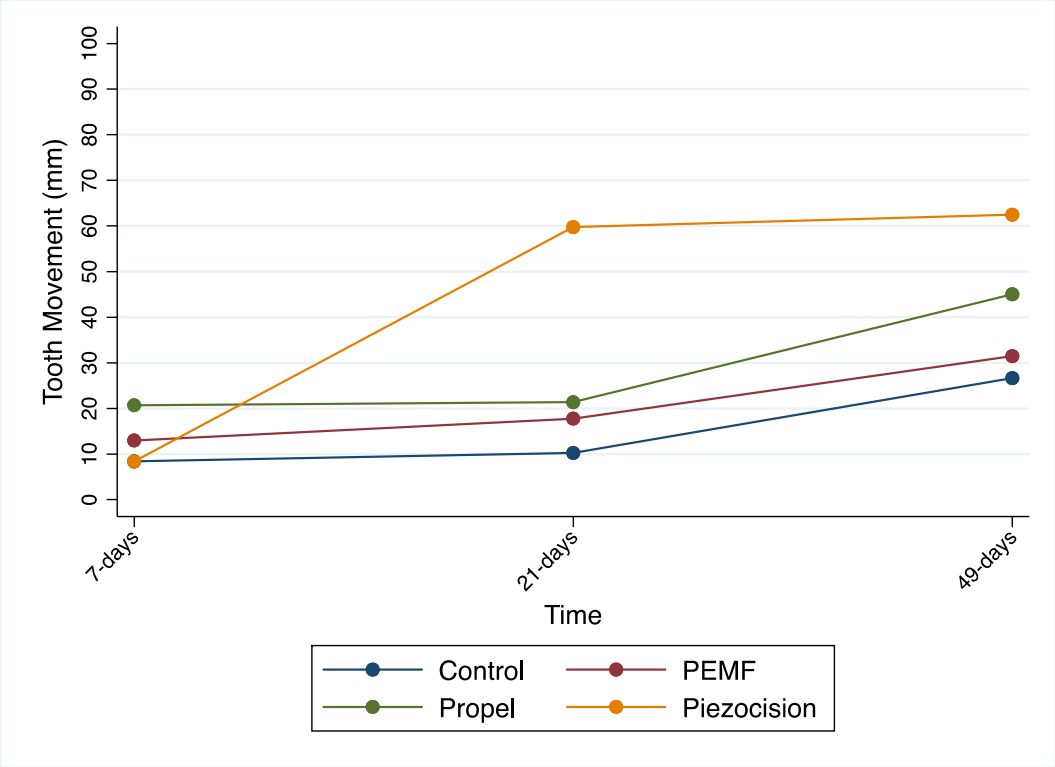


Figure 12: CBCT Intensity Difference (samples removed)

**Change in CBCT Tooth Distance**

We found a significant difference by time [$F$ (2, 29) = 15.57, $p < 0.001$, $\eta^2 = 55\%$], but no difference between groups ($p = 0.692$), or the interaction of group by time ($p = 0.636$). A post hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; no difference was found between day-7 and day-21 ($p = 0.046$) (Table 15).

| Day | Day | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| 21 | 7 | 0.84 | 0.01 | 1.67 | 0.046 |
| 49 | 7 | 2.08 | 1.31 | 2.84 | p < 0.001* |
| 49 | 21 | 1.23 | 0.40 | 2.06 | 0.003 |

Table 15: Pairwise Comparisons for CBCT Tooth Distance (samples removed)
*Statistically significant difference (p< 0.05).

| | Day 7 CBCT Tooth Distance | | | | Day 21 CBCT Tooth Distance | | | | Day 49 CBCT Tooth Distance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 0.20 | 0.20 | 0.00 | 0.40 | 0.70 | 0.36 | 0.40 | 1.10 | 2.13 | 1.51 | 0.50 | 3.60 |
| PEMF | 0.18 | 0.16 | 0.00 | 0.40 | 0.97 | 0.64 | 0.50 | 1.70 | 1.67 | 1.05 | 0.60 | 2.70 |
| Propel | 0.17 | 0.15 | 0.00 | 0.30 | 1.60 | 1.13 | 0.30 | 2.30 | 2.24 | 1.28 | 0.60 | 4.00 |
| Piezocision | 0.05 | 0.06 | 0.00 | 0.10 | 0.70 | 0.14 | 0.60 | 0.80 | 2.87 | 0.50 | 2.40 | 3.40 |

Table 16: Descriptive Statistics for CBCT Tooth Distance (samples removed)

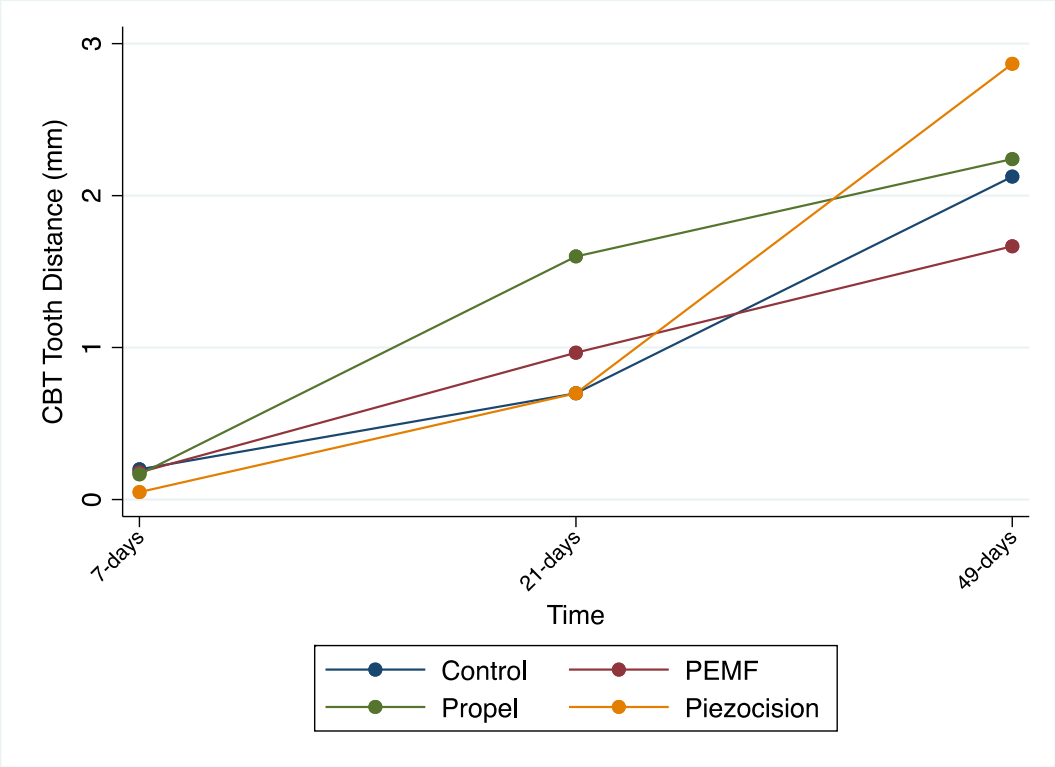


Figure 13: CBCT Tooth Distance (samples removed)

**Change in Posterior Shift**

To look for the change in CBCT tooth posterior shift we used a two-way ANOVA. The fixed effects were time (Day 7, Day 21, and day 49), group (control, PEMF, Propel, Piezocision), and the interaction of group by time. We found a significant difference by time [$F$ (2, 29) = 48.50, $p < 0.001$, $\eta^2 = 55\%$], but no difference between groups ($p = 0.163$), or the interaction of group by time ($p = 0.311$). A post hoc Tukey test showed that day-49 was significantly different from day-7 and day-21 at $p < 0.05$; day-21 was significantly different from day-7 at $p < 0.05$.

| | Day 7 CBCT Posterior Shift | | | | Day 21 CBCT Posterior Shift | | | | Day 49 CBCT Posterior Shift | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 0.07 | 0.12 | 0.00 | 0.20 | 0.20 | 0.17 | 0.00 | 0.30 | 0.55 | 0.06 | 0.50 | 0.60 |
| PEMF | 0.16 | 0.09 | 0.10 | 0.30 | 0.27 | 0.06 | 0.20 | 0.30 | 0.67 | 0.25 | 0.40 | 0.90 |
| Propel | 0.07 | 0.06 | 0.00 | 0.10 | 0.33 | 0.06 | 0.30 | 0.40 | 0.90 | 0.31 | 0.70 | 1.40 |
| Piezocision | 0.13 | 0.10 | 0.00 | 0.20 | 0.43 | 0.21 | 0.20 | 0.60 | 0.68 | 0.17 | 0.50 | 0.90 |

Table 17: Descriptive Statistics for Posterior Shift (samples removed)

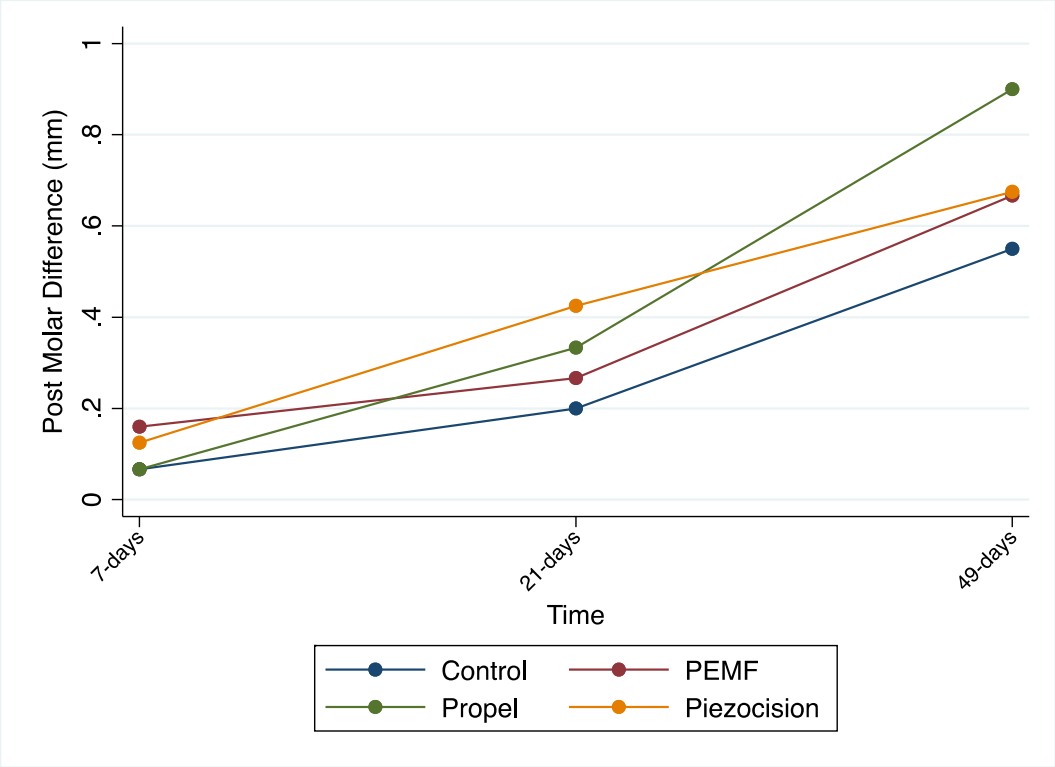


Figure 14: Posterior Shift. Posterior Molar Tooth Distance Difference (samples removed)

**Change in Histological Inter-Root Bone Percentage**

We found no significant difference by time ($p = 0.232$), a difference between groups [$F(3, 29) = 3.56$, $p = 0.025$, $\eta^2 = 19\%$], but no significant interaction of group by time ($p = 0.193$). A post hoc Tukey test showed that Piezocision was significantly different from the control at $p < 0.05$ (Table 18).

| Group | Group | Difference | Lower 95% CI | Upper 95% CI | p-value |
|---|---|---|---|---|---|
| PEMF | Control | 4.40 | -7.14 | 15.94 | 0.732 |
| Propel | Control | 8.86 | -2.68 | 20.40 | 0.181 |
| Piezocision | Control | 12.71 | 1.56 | 23.87 | 0.021* |
| Propel | PEMF | 4.46 | -6.92 | 15.84 | 0.715 |
| Piezocision | PEMF | 8.31 | -2.68 | 19.30 | 0.192 |
| Piezocision | PEMF | 3.85 | -7.14 | 14.84 | 0.778 |

Table 18: Pairwise Comparison for Histological Inter-Root Bone Percentage (samples removed)
*Statistically significant difference (p<0.05)

| | Day 7 Bone Percentage | | | | Day 21 Bone Percentage | | | | Day 49 Bone Percentage | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 64.43 | 13.38 | 49.30 | 74.70 | 67.20 | 3.22 | 63.80 | 70.20 | 75.05 | 6.59 | 67.80 | 83.40 |
| PEMF | 63.18 | 12.17 | 41.80 | 72.10 | 61.37 | 7.56 | 57.00 | 70.10 | 74.67 | 6.10 | 69.50 | 81.40 |
| Propel | 51.87 | 13.63 | 40.30 | 66.90 | 62.13 | 7.91 | 54.40 | 70.20 | 68.14 | 7.44 | 59.30 | 79.60 |
| Piezocision | 59.48 | 9.49 | 46.10 | 66.90 | 50.98 | 9.64 | 45.30 | 65.40 | 62.03 | 13.53 | 44.30 | 76.30 |

| | Day 7 Bone Percentage Control | | | | Day 21 Bone Percentage Control | | | | Day 49 Bone Percentage Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 80.57 | 2.97 | 77.30 | 83.10 | 71.93 | 7.20 | 64.70 | 79.10 | 79.78 | 7.59 | 68.90 | 86.10 |
| PEMF | 77.96 | 2.45 | 74.60 | 80.70 | 71.97 | 7.70 | 63.80 | 79.10 | 85.70 | 2.69 | 82.60 | 87.40 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Propel | 76.17 | 8.81 | 66.80 | 84.30 | 76.43 | 4.40 | 73.00 | 81.40 | 77.96 | 5.37 | 69.30 | 82.70 |
| Piezocision | 77.03 | 4.83 | 70.30 | 81.00 | 81.63 | 3.66 | 77.50 | 85.00 | 78.75 | 5.97 | 70.50 | 84.70 |

| | Day 7 Bone Percentage Change | | | | Day 21 Bone Percentage Change | | | | Day 49 Bone Percentage Change | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | Min | Max | M | SD | Min | Max | M | SD | Min | Max |
| Control | 16.17 | 10.41 | 8.40 | 28.00 | 4.70 | 4.01 | 0.90 | 8.90 | 4.70 | 7.82 | -3.70 | 12.80 |
| PEMF | 14.80 | 12.99 | 6.90 | 37.90 | 9.37 | 8.06 | 2.90 | 18.40 | 14.60 | 9.44 | 6.00 | 24.70 |
| Propel | 24.27 | 6.09 | 17.40 | 29.00 | 14.30 | 8.33 | 4.70 | 19.60 | 13.58 | 9.08 | 1.60 | 21.70 |
| Piezocision | 17.60 | 6.20 | 10.00 | 24.20 | 30.65 | 11.25 | 14.30 | 38.40 | 15.45 | 12.61 | -0.90 | 26.20 |

Table 19: Descriptive Statistics for Histological Inter-Root Bone Percentage (samples removed)

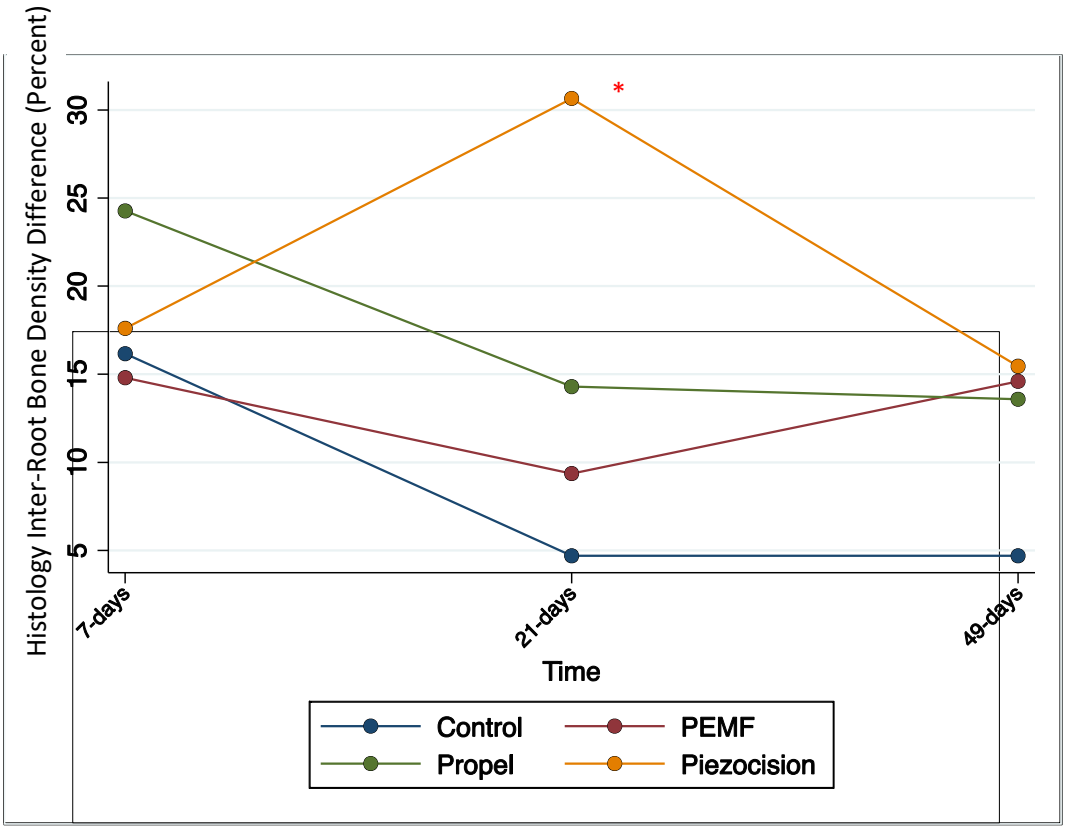

Figure 15: Histological Inter-Root Bone Density Difference (samples removed)
*Statistically significant difference ($p < 0.05$).

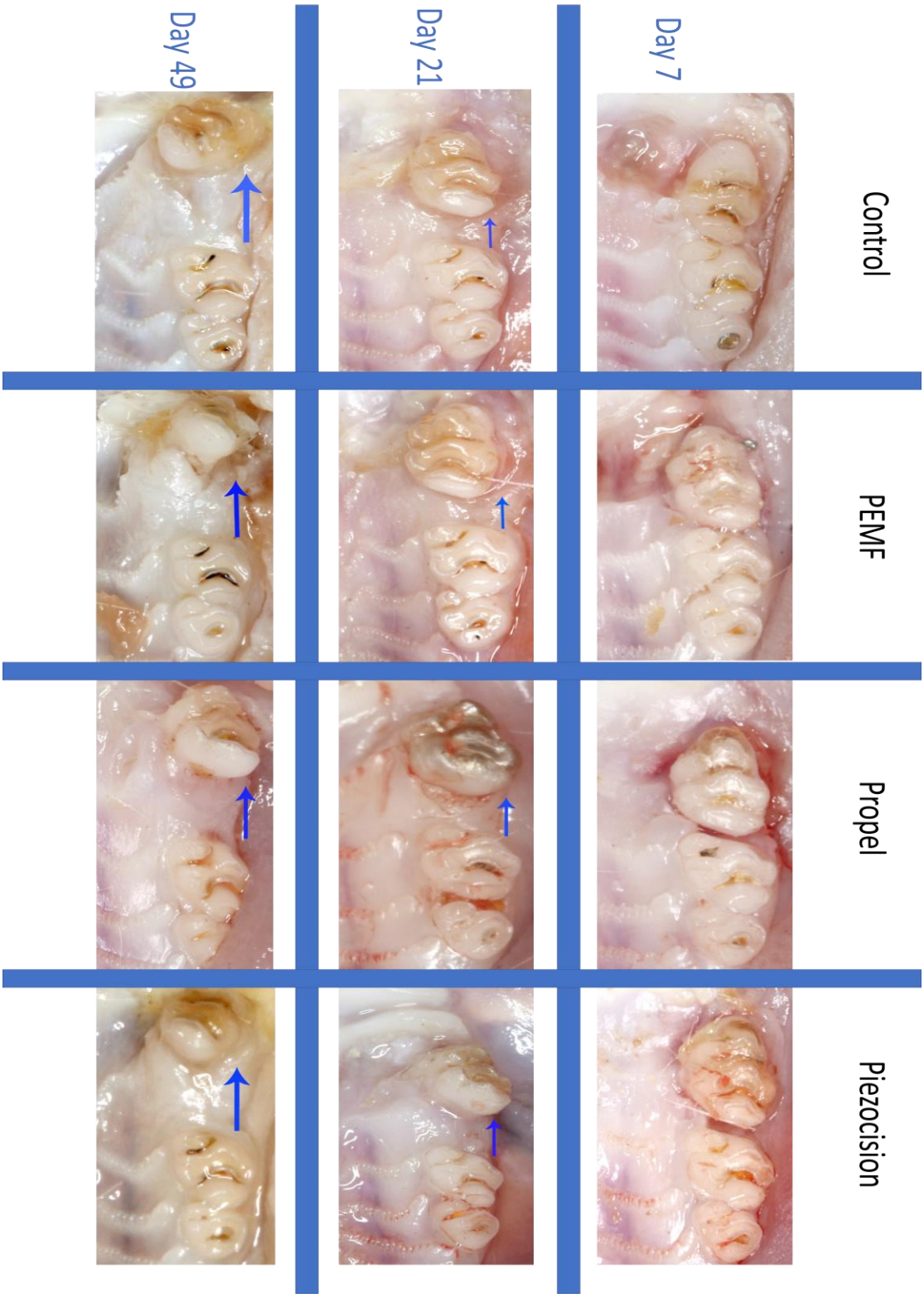


Figure 16: Clinical pictures of each group at each time point.

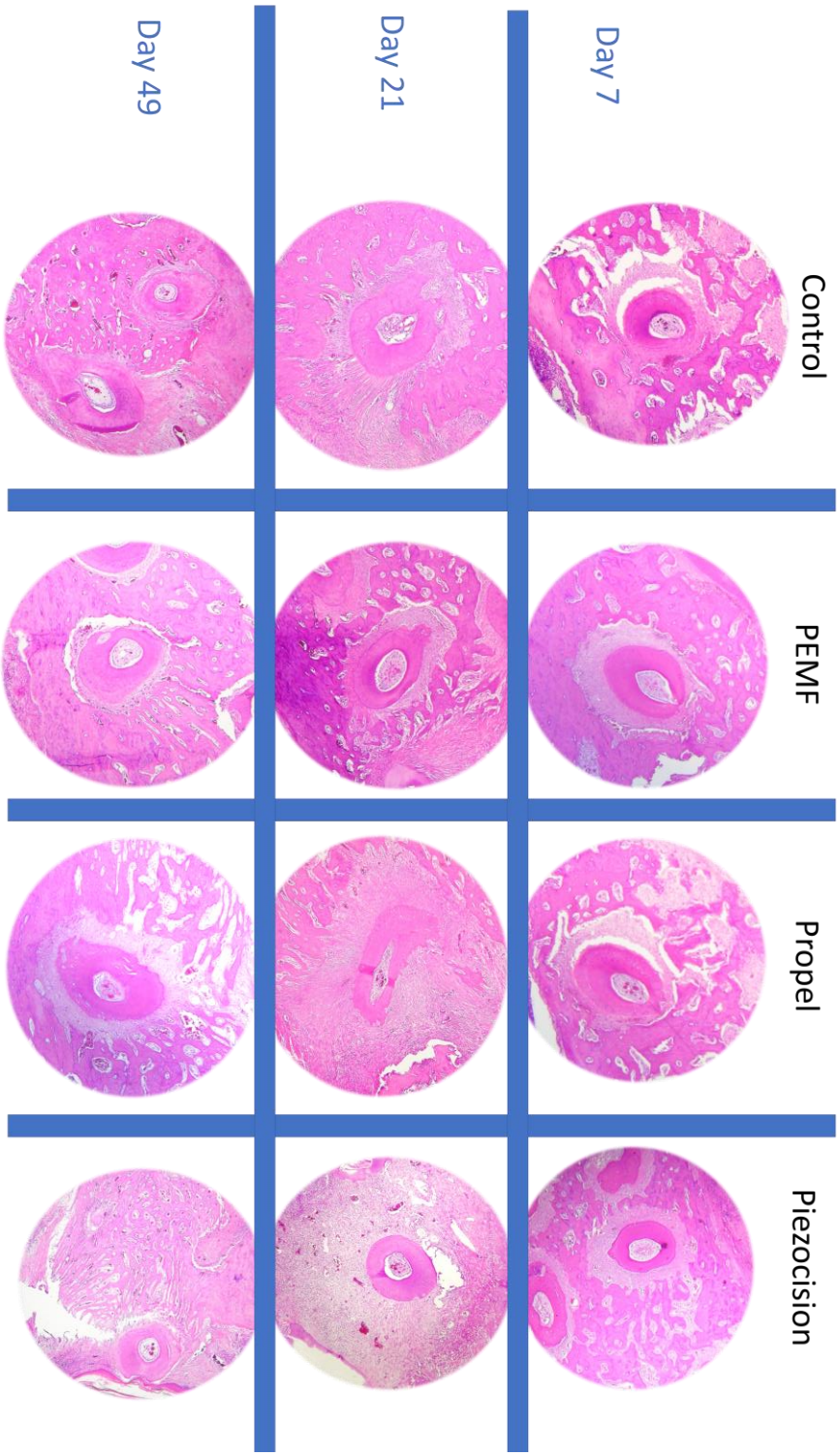


Figure 17: Histological cross-sections of each group at each time point (Magnification 10x).

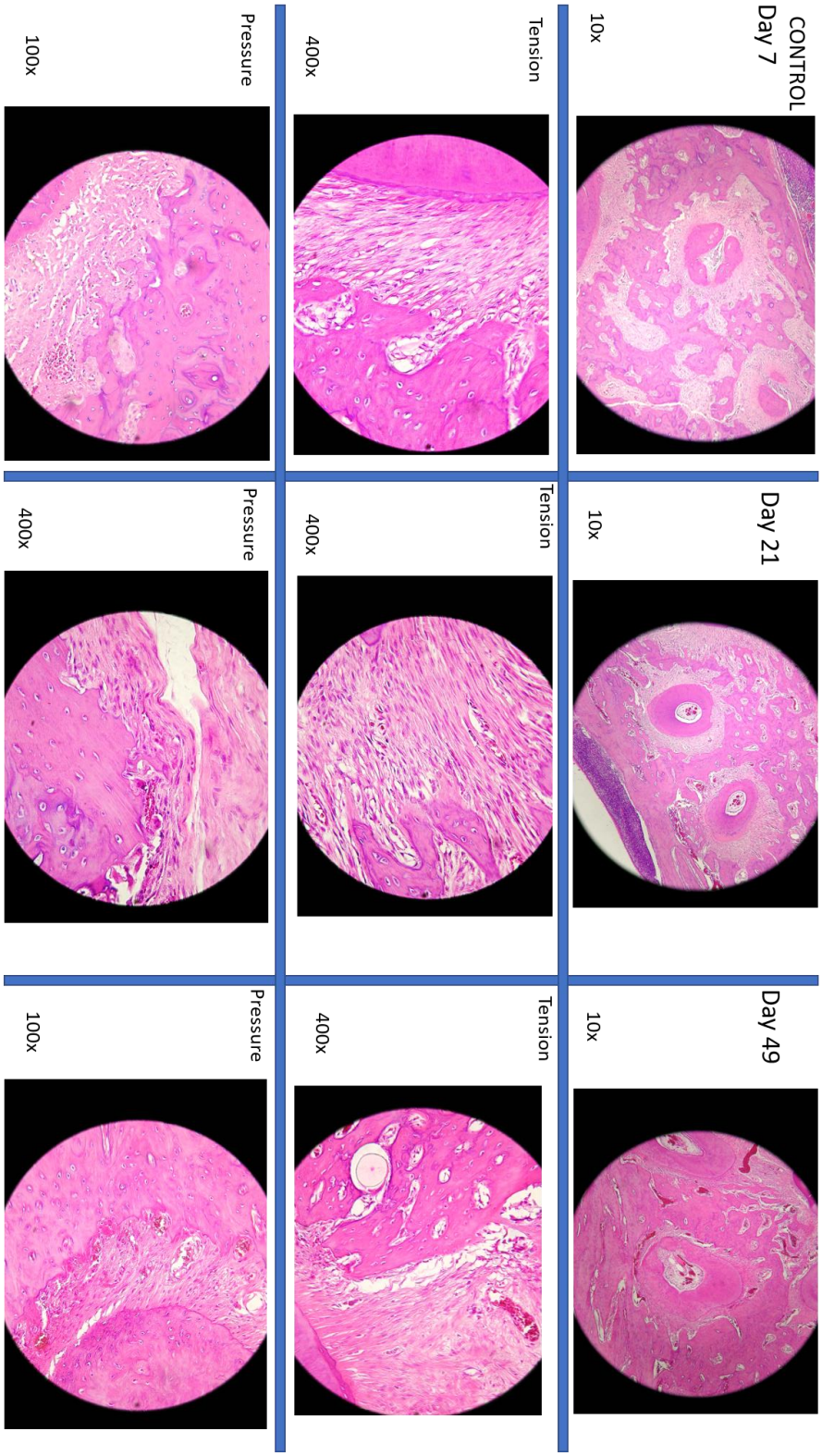


Figure 18: Histological cross-sections of Control Group at each time point.

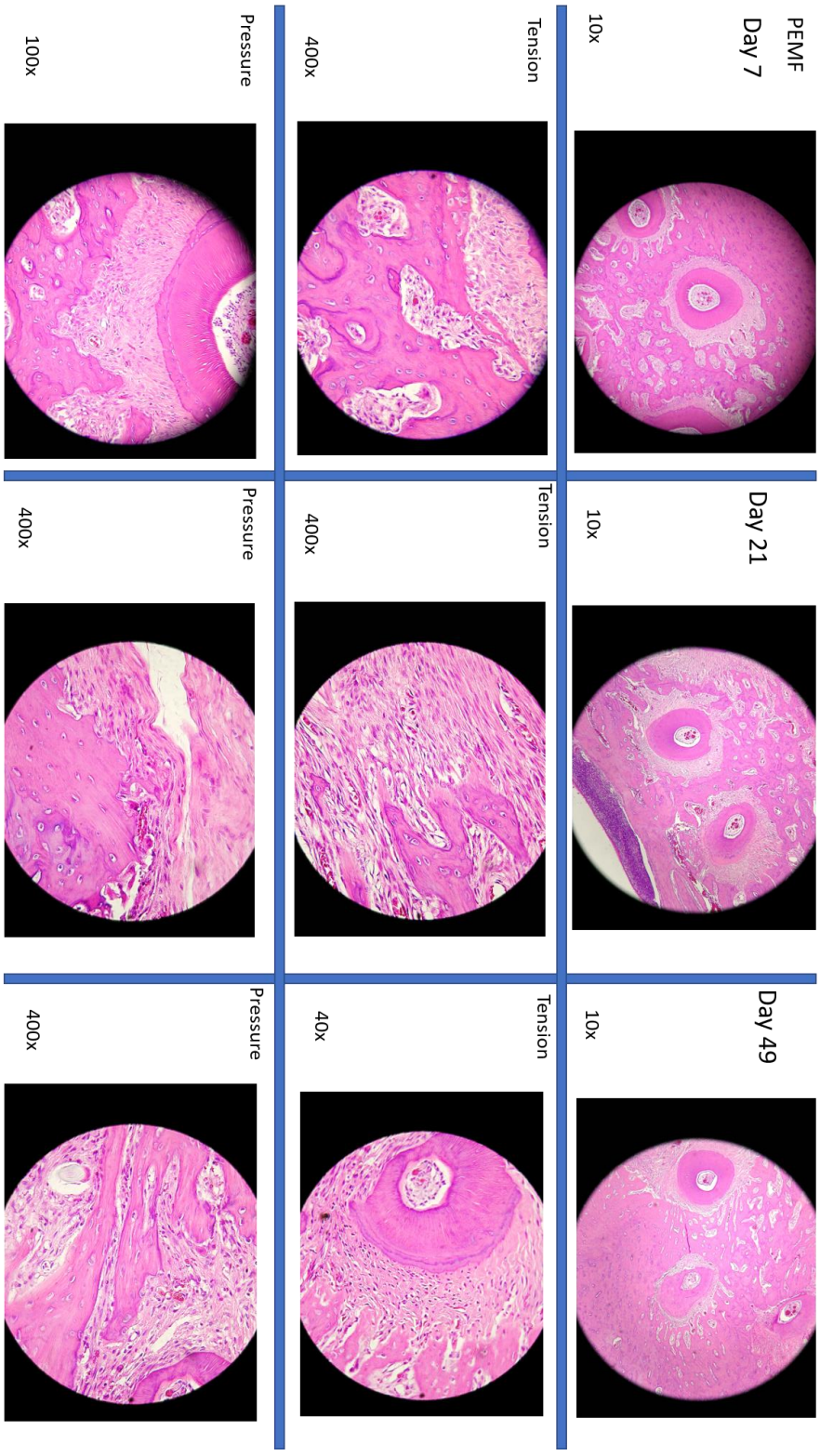


Figure 19: Histological cross-sections of PEMF Group at each time point.

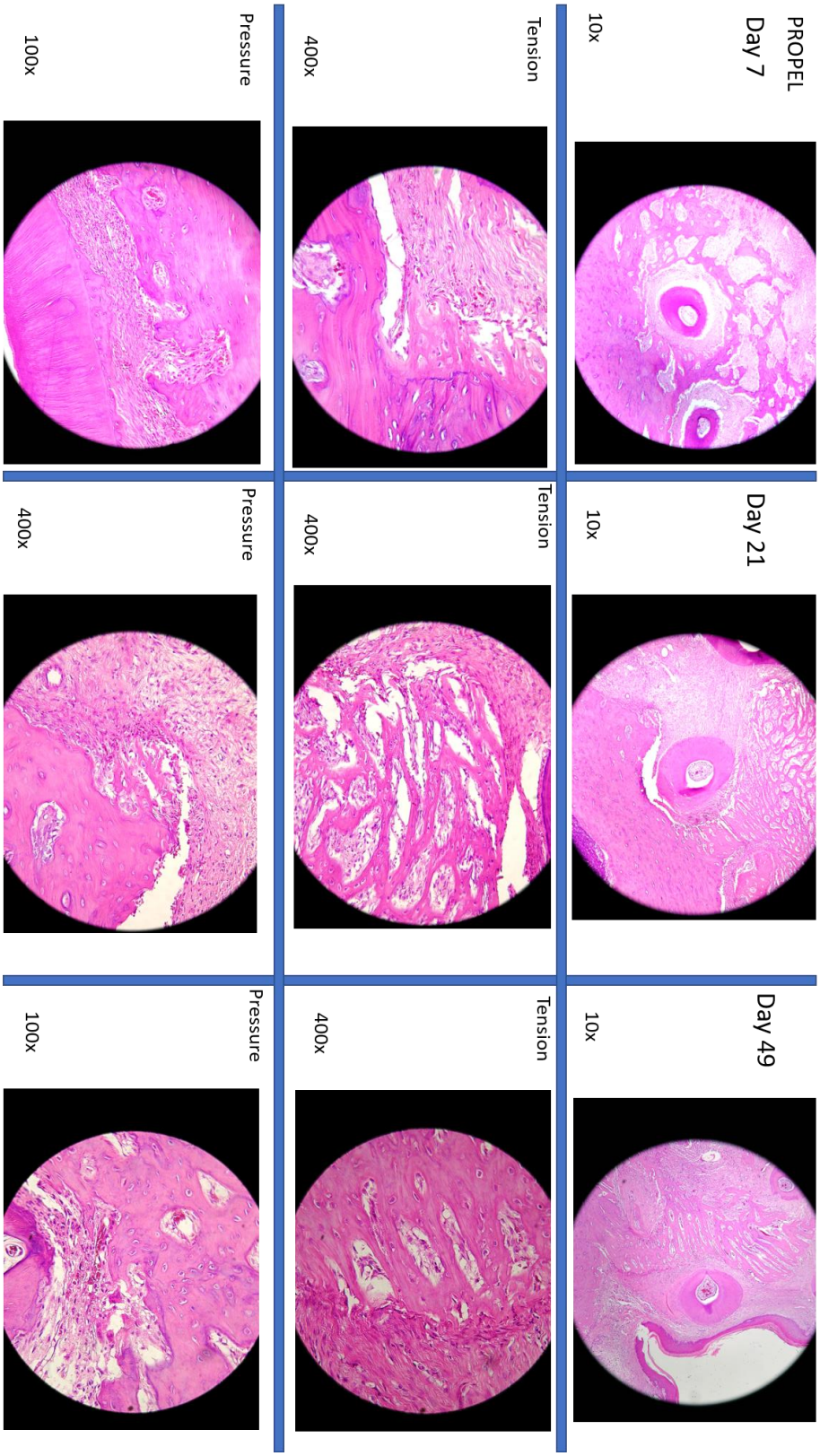


Figure 20: Histological cross-sections of Propel Group at each time point.

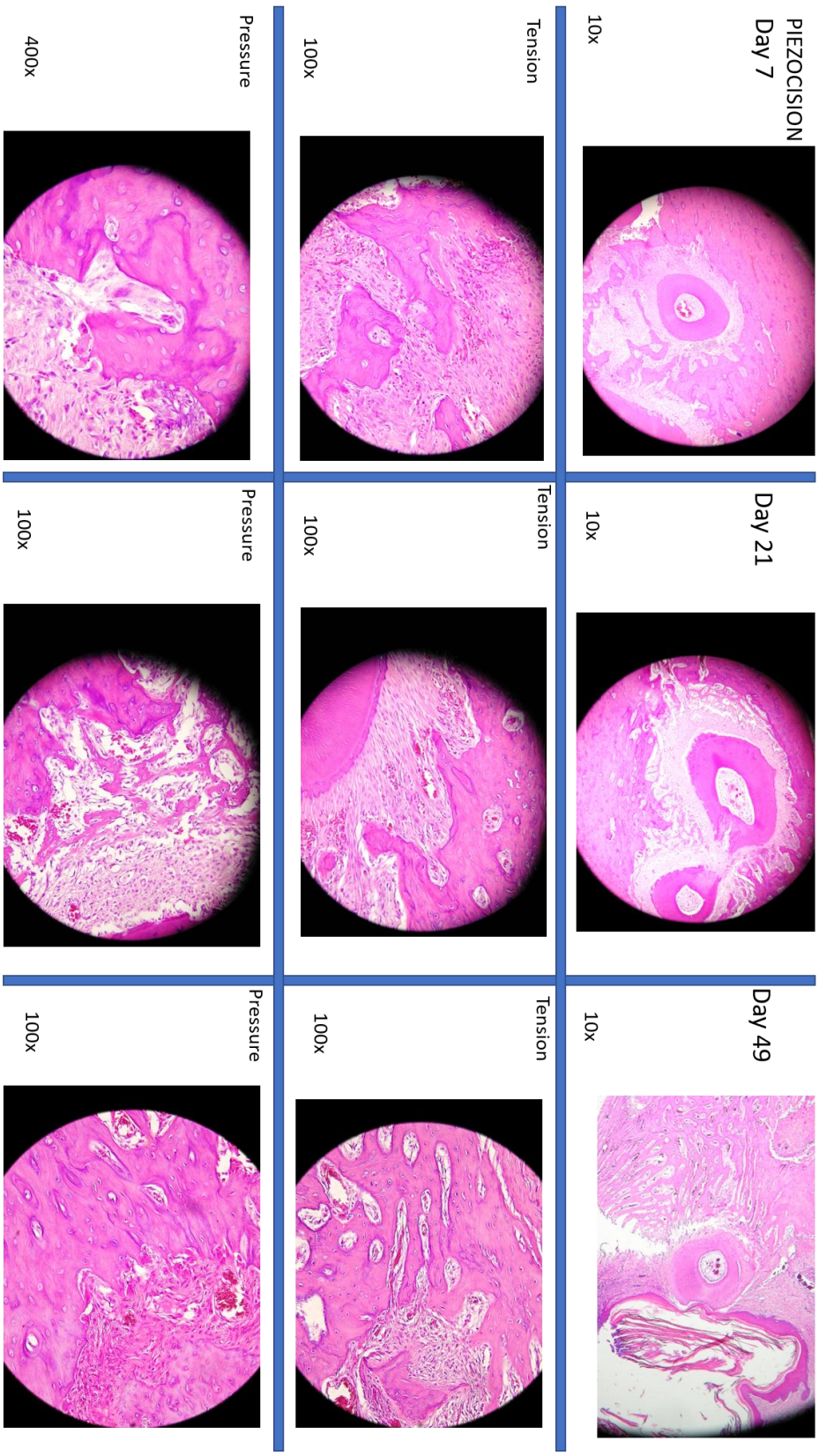


Figure 21: Histological cross-sections of Piezocision Group at each time point.

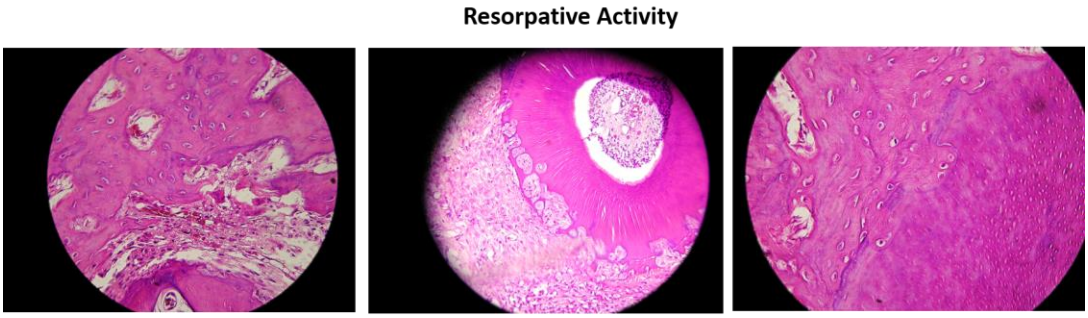


Figure 22: Histological cross-sections demonstrating resorptive activity.

A) Multinucleated osteoclasts can be visualized within resorption lacunae in denser regions of intra-radicular bone. From a 7 day Piezocision sample. B) Excessive resorption leading to root resorption. Often depicted in areas of pressure in many samples. This aggressive resorption was noted in a 21 day Piezocision sample. C)Ankylosis was noted in several samples in several groups. From a 21 day control group.

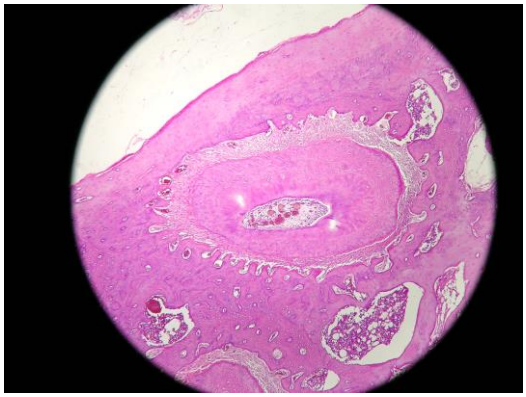

Figure 23: Histological cross-sections demonstrating capillary lacunae.

Around the PDL space on the alveolar side, indentations can be noted all along. These regions often contain larger vessels. As tooth movement begins these regions begin to expand and become continuous with the enlarging PDL space. From a non-test side molar.

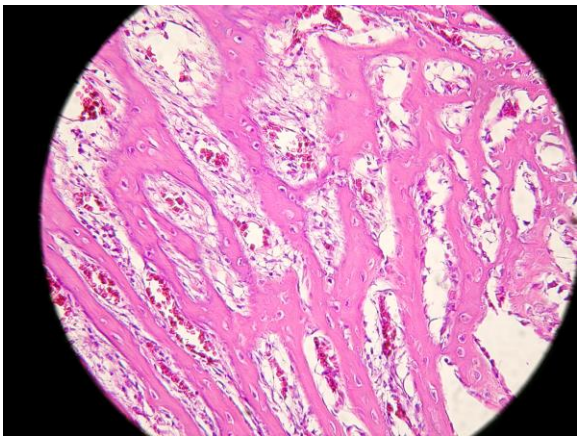

Figure 24: Histological cross-sections demonstrating unique bone remodeling.

Upon reconstitution of the alveolus at 49 day groups that had significant movement, the bone forms in these elongated patterns. Young bone is interposed with long marrow spaces with newly developing vessels. They often are directed in the same path as the vector of tooth movement. Due to the lighter staining young bone, the PDL fibers often appear continuous with the developing bone. From a Day 49 Propel group.

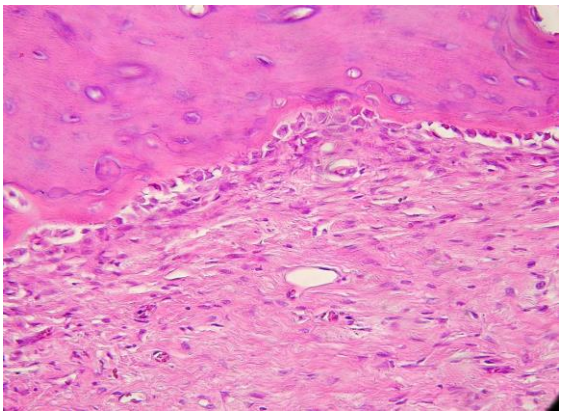

Figure 25: Histological cross-sections demonstrating osteoblastic activity.

Osteoblasts can be seen lining the bone and adding a new layer of osteoid in a region of tension within the PDL. Incremental lines can also be depicted. From day 49 Piezocision group.

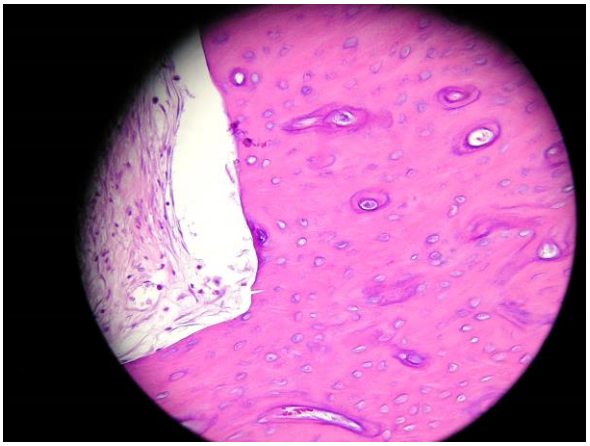

Figure 26: Histological cross-sections demonstrating bone adjacent to Piezocision decortication site.

Decortications were best visualized in 7 day samples. Lacunae directly adjacent to the injury appear empty as osteocytes have likely been lost. The depth of empty lacunae is limited in this Piezocision sample. The preservation of osteocytes adjacent to injury site may result in a faster response.

# Discussion

## The Rat Model

The model used in this study has been derived from many similar studies(Baloul et al , Dibart et al 2013). Several limitations were noted in this particular model including mesialization into basal bone, force of the closed coil spring, and the limited time interval in which tooth movement can be observed.

During the mesialization process several factors were noted as potential variables that could affect the outcome of movement. For example, if the closed coil spring is situated on different aspects of the tooth, then the resulting positioning of the tooth can be affected. Rotation and tilting may occur and skew the clinical measurements.

Tilting of anchorage teeth can occur when anchorage is not sufficient to move the desired teeth. In this rodent test, the anchorage of the incisors may have not been sufficient to mesialize the test molar through dense cortical bone. Clinical backwards tilting of the incisors was noted in several samples but was not documented numerically. Relative anchorage has been suggested to change when the RAP effect is induced in regions of desired tooth movement (Dibart et al. 2011). As the transient osteopenia allows for less resistance to tooth movement in the region of the controlled injury, the anchorage tooth has less reciprocal force applied to it. This results in a relative increase in anchorage. In this model, the relative increase in anchorage could have resulted in less tilting of the anchoring incisor. The test molar would be moving in softer bone resulting in less pulling force on the incisors. The control group may cause more tilting of the

incisors. This would result in less interdental space and an overestimation of the amount of the amount of tooth movement in the control group.

The same concepts apply to rotational forces. The mesial region of the test molar is directly proximal to a region of horizontally deficient dense cortical bone. Upon greater forces the cortical bone may not remodel fast enough to facilitate bodily movement of the test molar. The spring in most groups was located at some portion of the palatal aspect of the test molar. If the closed coiled spring was fixated further distally on the palatal, the slow remodeling mesial bone may redirect the rotation of the tooth. This could theoretically result in rotational forces sending the distal palatal rotating further mesial in conjunction with tilting. This again may skew clinical measurements. The significance of an interventions ability to reduce undesired tilting and rotating during movement of teeth through inadequate bone has yet to be investigated in this orthodontic model. Further investigations need to be conducted considering the relative potential of bone quality and number of rotational/tilting vectors produced under similar orthodontic applications in this rodent model.

Another potential issue with mesialization in this model is the presence of basal bone mesial to the first molar. The test molar traveled nearly 2 times the size of the molar, which is a relatively far distance as most molar movements in humans do not involve closing a two-molar space. The bone directly mesial to the test molar was almost entirely dense type 1 bone, and the edentulous span was never genetically or phenotypically determined to harbor teeth. Essentially, this can be considered an atypical movement relative to the space closures we test in humans and larger animals. Most movements performed in a typical clinical scenario is within the alveolar housing. This specific edentulous ridge area in rats has histological resemblance of basal bone. An absolute lack of marrow spaces is noted with defined and wide lacunae. Despite this bone quality, as teeth were moved into this zone, the periodontium appears to continue with the tooth. So, this

may only be relative limitation that is dependent on the variables such as force and tooth durability. The ability of tooth movement through bone that is not genetically predisposed to harboring a tooth has not been adequately investigated in the literature. To develop a RAP affect it may be important to have some underlying cancellous bone. Since the bone is very dense in the edentulous site of mesialization, any attempt to create an injury would not expose cancellous bone as is seen on the distal of the test molar. Similar studies on accelerated orthodontics suggested that a mesial and distal decortication could be performed on the test molar (Dibart et al. 2013, Teixiera et al. 2010). These studies performed the mesial decortications with the pretense that the cortical bone would not be 0.5mm thick. On the facial and palatal of the dentate region, this depth would sufficiently penetrate the cortical bone (Ibrahim et al. 2017). Histological evidence in this study shows a relatively deep layer of dense cortical bone, nearly the full thickness of the ridge on the mesial.

The cancellous bone plays a vital role in supplying the cellular activity to remodel the injured bone. With the limited vascular supply in the edentulous site, the mesial interventions performed may have a limited effect on the mesialization of the molars. Thus, this model may have limited effectiveness for observing clinical differences amongst techniques. This may also explain the limited clinical differences observed amongst the groups in this study. Future rat studies utilizing mesialization may be improved by extracting the first molar and mesializing the second molar after the first molar site has healed. This method would allow for mesialization through a region of cancellous alveolar bone. This would also allow for a more realistic simulation of the RAP effect upon surgical and non-surgical accelerated orthodontic intervention. Considering the above variables in conjunction with sample size, it could be suggested that this model does not provide sufficient data to make clinical rate conclusions. It has been suggested that the rat model provides certain advantages in observing early tooth movement chemical and

histological changes rather than quantity-based changes, such as rate and amount of movement (Ibrahim et al. 2017).

An additional factor that may be important to note in this model is the force of the appliance. Previous rat accelerated orthodontic models were able to demonstrate a statistically significant difference between control and intervention (Alikhani et al. 2015, Dibart et al. 2013). Potential major differences were the measured time durations were shorter and the orthodontic forces used were 25g. This may suggest the mesialization force of a 50g force NiTi closed coil spring has the potential to mesialize under any condition or intervention. Prior studies on rat models utilizing a 50g closed coil spring demonstrated sufficient mesialization capabilities with no significant difference in resulting pathological changes as compared to 25g (Gonzalez et al. 2008). Prior studies utilizing accelerated orthodontic techniques with 25g closed coil springs reported movement results at similar times of approximately 2-3mm by day 42 as our study (Baloul et al. 2011). The slightly more movement seen in our study may be either the result of utilizing a 50g force closed coil spring or the extended duration of the study of 49 days vs 42 days. A micro-osteoperforation RAP induction model used a 50g force spring and observed similar results (Teixeira et al. 2010). The mean amount of teeth movement noted at day 28 (0.6mm) resembles the mean amounts in our study at day 21 (0.7-1.6mm). With that in mind these studies were capable of demonstrating a statistical significance. Applying an intervention may affect rate at lower forces, but it is possible that rate may only be minimally affected at higher forces. At extremely higher forces the resorption front from the RAP created may not be sufficient to prevent compression on the pressure side. Thus, necrosis could still occur resulting in delayed movement. The amount of undermining resorption may also be affected by the RAP induction. If over-compression of the pressure side occurs, the native increase in resorptive cytokines and cells may be exacerbated by the influx of catabolic activity from the RAP effect. This could increase the

risk for pathological tooth and periodontium changes. On the other hand, the bone turnover abilities of the RAP effect could expedite the undermining resorption and reduce the density on the pressure side. Hence, the catabolic based pathology would be less likely to occur. To better understand this scenario, further investigations into the RAP effect in areas of extreme pressure need to be investigated including cytokine profile and cellular activity.

The final time point used in this study was 49 days. The amount of movement seen in all groups at 49 days suggests possible complete closure of the closed coiled springs. Thus, complete closure may have occurred between day 21 and 49 for different groups. But by day 49 all groups were able to show near completion of orthodontic movement with 50g of movement. The exact moment of tooth movement completion may have been missed between these two intervals limiting the potential of understanding which technique results in faster tooth movement. It has been suggested that the rodent orthodontic is only suitable for several weeks rather then 7 weeks (Ibrahim et al. 2017). Additionally, the clinical and histological observations demonstrate that the test tooth is moved outside the arch by the 49 day time point. This would result in undesired tilting and attachment loss. Future studies may consider more time intervals under 49 days.

Lastly, the sample size may have affected the statistical power within this study. Several samples did require repair of appliance during the study. Also a few samples in the Piezocision group had complete avulsion of the test tooth prior to completion and were included at the closest time point as they were within 48hrs of a sacrifice time point. The groups with avulsions beyond time points were not included in statistical analysis of clinical movements. The Piezocision group ended with a smaller sample size then the control group (n=3 vs. n=5) due to avulsion. This could have directly resulted in skewed data. The clinical measuring system was also exposed to several variables. These include human error and growth anomalies. These could explain certain negative movement values measured at the 21-day mark.

## Measurement Techniques

The clinical tooth measurements presented with limitations. No statistical significance could be derived from this method. The measurements were taken as the initial step prior to any interventions. The measurements were taken from the most proximal gingival margin location relative to the edentulous space. As tilting occurs the region of the tooth that was originally used for measuring shifts further subgingivally. Hence, the exact baseline measurement site on the test molar cannot be reached and an area slightly more mesial was used to measure the amount of change in mesialization. Despite the relatively large anchorage potential of the incisors, the incisors may have tipped distally. The distal tipping would reduce the space measured and result in a larger relative distance closure calculation. More incisor tilting would overestimate the amount of tooth movement.

The mesialization of the test molar was also measured radiographically from the mesial contact point of the second molar to the nearest point of the test molar. This method reduces certain human errors in measurement. Utilizing a cone beam image, teeth could be aligned to produce relatively similar points of measurements. All contacts between the first molar and second molar were in direct contact at baseline, and thus any measured changes were a result of tooth movement. This measurement could be done to the nearest tenth of mm. This minimizes measurement issues with tilting since the incisors are not involved in the measurement. Also, issues with rotating are slightly minimized as an indirect device does not need to be applied to areas with altered soft tissue dimensions. The sample size issue was still applied to this measurement method as teeth were avulsed during handling of samples at certain time intervals, primarily in the Piezocision and Propel groups.

The CBCT analysis for bone density also presented with certain advantages and disadvantages. The benefits of utilizing CBCT imaging included the ability to observe the relatively same transverse plane on the test molar. Histological observations would be limited by the slide preparation which may have slight angulation differences. A potential limitation could have been derived from the resolution of the cone beam images. Micro CT analysis would contain greater pertinent data at the scale of the rodent model. Additionally, the selected field for observing radiodensity changes at different time points may not be ideal for eliciting differences as much of the anatomy is different at each time point. The ridge is thinner when the tooth moves to day 49. Thus, the potential for changes in bone density is limited as there is less potential anatomy to analyze in this region. Considering the various anatomies, it was beneficial to compare all samples to their own contralateral side.

Histological bone density measurements also presented with limitations. Although the grid between roots created a relatively uniform space to measure bone quality, the roots would be included in the measurement. Some samples would have less distinct cemental layers and higher fiber density then others. This would create false positives of bone when adjusting threshold levels. Despite this potential limitation, the effects were limited to a minor portion of the measured field and did not isolate into any specific group.

### Bone Density and Tooth Movement

Bone density differences noted in this study shed light into potential differences in these accelerated orthodontic techniques. Piezocision showed a statistically greater reduction of radio-density reduction than the control group. The decortication groups tended to have a greater reduction in radiographic bone density at days 21 and 49 as compared to other groups. Especially at day 49 the radio-density surrounding the test molar is significantly reduced in Propel and Piezocision groups. The relative decreased radiographic bone density suggests possible

reduced bone mineralization. This may be the result of the RAP effect creating a localized osteopenia in the region of the test molar. Thus, decortication groups could have an osseous environment more conducive for accelerated orthodontic tooth movement. The relative decrease in radiographic bone density of the Piezocision group does coincide with the higher radiographic tooth movement. As the density of the bone decreases it may relate to relative amount of tooth movement. The trend of bone density change for the Piezocision group appears to vary from the other groups. Bone density in most groups appear to gradually decrease from day 7 to 21 with a steeper decrease at day 49. The Piezocision group decreases steeply from day 7 to 21 and gradually decreases from day 21 to 49 resembling a more logistic curve. This may suggest that the RAP effect brings bone remodeling earlier and greater with Piezocision then other modes of accelerated orthodontics. Propel also appears to have a potential for significant bone remodeling but may have a limited potential without an initial boost in osteoclastic activity.

Research is limited in utilizing CBCTs to analyze bone density changes around orthodontically moved rat molars. Using histological and Micro CT analysis from prior studies, the trend of continual bone mineralization reduction is consistent with studies observing Piezocision and micro-osteoperforations (Dibart et al. 2011, Teixeira et al. 2010). In a study by Dibart et al. in 2011, the histological evidence suggests bone remineralization at time points beyond 49 days. Although the CBCT findings in this study suggest a reduction in mineralization at day 49, the histological data coincides with prior studies demonstrating higher levels of bone density at day 49. Although CBCT imaging often provides adequate accuracy of bone density, bone density changes often take longer to visualize radiographically (Akesson et al. 1992, Grimard et al. 2009). As osteoclasts begin to resorb bone and reduce mineralization, the radiographic evidence of demineralization is delayed. Thus, the radiographic density results may possibly underplay the amount of the demineralization occurring. Histological evidence does not directly correlate with

the density changes, as well. The day 49 histological samples tend to have a higher mean bone presence between the roots then day 21 in the decortication groups. The bone present at day 49 may not be completely calcified and/or may be immature. The mineralization of this newly formed bone may not be substantial yet to demonstrate a radiographic rebound of sound alveolar bone.

Bone has several stages of development. In earlier stages, the bone is an intricate collagen matrix yet to be filled with calcium phosphate minerals (Sodek and Mckee 2000). As this osteoid begins to receive minerals, the relative radio-opacity would not be that of mature bone. As the mineral content increases and organizes into a crystalline structure, the radio-opacity increases. So, bone may begin to take form histologically, but until the mineralization of the osteoid is complete the relative radio-opacity will not translate into dense bone radiographically. This could explain the disconnect between radiographic and histological densities at day 21 and day 49. Since the potential for more demineralization and collagen matrix alterations are noted in the decortication groups at day 21, a greater amount of time is required to re-achieve mature dense bone in the Piezocision and Propel groups. Assuming mature bone is that which contains adequate remineralization, the Piezocision group may be showing signs of delayed remineralization. This would help explain the trend of the lower radiographic bone density at day 49. It also correlates with the decreased bone volume noted at day 21 in the histological analysis. Observational analysis showed signs of rampant osteoclastic activity at day 21 in the surrounding periodontium. Despite the amount of histologic changes noted in several samples, the day 21 radio-opacity had not reflected as significant of a bone density change. The radiographic evidence of bone loss takes longer to become evident than actual attachment loss. The radiographic bone density at day 49 more likely resembles the histological catabolic osseous breakdown at day 21. The crystalline structure may be broken down, but calcium and phosphate deposits may have not yet exited the

area at day 21. Some levels of calcium may be shunted away from the site via the vasculature, but the physiology may be dictating the future need for the calcium in the immediate area.

Often a breakdown of the crystalline structure is dictated by hormones, such as parathyroid hormone, to increase blood calcium levels (Sodek and Mckee 2000). In the scenario of the RAP effect, blood levels may be adequate, and a negative feedback loop would prevent the excessive efflux of calcium from the site of osteoclastic activity (Frost 1983). Additionally, the absence of a pathogenic source of osteoclastic mediating signalers could also limit the shunting of calcium from the region of tooth movement. On the other hand, it can be speculated that the RAP effect could result in excessive continual removal of mineralization and make the reversal process prolonged. The physiology of calcium influx/efflux from a RAP induced region may not follow the same principles as other bone breakdown events.

Histological bone density measurements also demonstrated a statistical significance in the Piezocision group at day 21. All the groups had relatively similar bone density reductions at day 7, but by day 21 the densities began to vary. A distinct trend between the decortications groups and the control/PEMF groups could be noted again. The decortication groups had a slight decrease or stayed similar in bone density at day 21 compared to day 7. The PEMF and control groups had a decrease in bone density compared to its contralateral side. The decortication groups retained their relative decrease in bone at day 21 that started at day 7. But the Piezocision group trended towards having a greater amount of bone density reduction. This has been emulated in previous histological studies evaluating Piezocision, showing nearly complete loss of intra-radicular bone density (Baloul et al. 2011, Dibart et al. 2013). Thus, the bone may have undergone further resorption to facilitate orthodontic movement. This is likely a result of the RAP effect induced by these interventions. Studies evaluating micro-osteoperforation induced intra-radicular density changes via Micro CT have shown significantly lower density levels (33%)

(Teixeira et al. 2010). Although Micro CT values cannot be directly compared to histomorphometric data, this suggests a drop-in mineralization in a similar fashion as seen in our histological measurements.

The bone density of the control group and PEMF group began to trend towards baseline at day 49. This may indicate a possible decrease in tooth movement. The radiographic tooth movement levels support this trend as well. Tooth movement in bone that has not undergone resorption may take longer or possibly result in over compression of the pressure side PDL (Gonzales et al. 2003). The orthodontic force will move the tooth despite the presence of bone. If bone is too dense, the PDL will compress leading to a series of events to resorb the bone (Feller at el. 2015). Too much compression leads to too much necrosis. As mentioned earlier, this may result in pathological changes in the root or the attachment (Gonzales et al. 2003). The groups that retained a certain level of bone density reduction have less potential chances of over compressing the PDL space. Denser sites would still require remodeling, which could delay the rate of tooth movement. Some samples in the Piezocision and Propel groups demonstrated a near complete loss of bone density histologically at day 21. These teeth could be considered to be highly mobile. Several of these samples were correlated to the samples that had avulsed teeth during sectioning of the jaws. These samples would have limited issues in rate of tooth movement or pathological changes due to over compression of the PDL space. Avulsion of the decortication group teeth at day 21 is likely a result of the local demineralization. It has been suggested that long term evaluations on tooth movement in rats can result in tooth avulsion as teeth are moved out of the alveolus (Ibrahim et al. 2017). In this specific scenario the teeth are still in the alveolus at time of avulsion. This is confirmed with histology of these samples showing a front of alveolar bone outside the zone of demineralization. Also, the teeth that did complete movement by day

49 were less often avulsed then those at day 21, which is when the demineralization due to RAP would be the greatest.

At 49, a similar trend as noted in day 21 is seen with a continuation of bone density difference in the decortication groups. The control and PEMF group both had a slight decrease in density. At this point, the bone resorption may have begun to reach a level to facilitate tooth movement. This could explain the resultant amount of tooth movement accomplished by these groups at day 49. The clinical tooth movements reached higher levels by day 49 matching closely with the decortication groups. Since all groups completed tooth movement by day 49, it is possible that tooth movement measurements between 21 and 49 days may have shown the results of a lag phase. Since the bone density remained constantly low in decortication groups, they may have been able to maintain a rate of tooth movement through the 21 days and reached completion prior to the 49 days mark. The PEMF and control groups may have had to take longer to remodel bone to reach the same amount of tooth movement. Although the movement may have been completed by day 49, it is possible that this would have delayed the movement completion beyond the time it took the decortication groups (Figure 24). The consistency of the bone at day 49 in several samples resembled a younger form of bone. The collagen fibers that were laid down to form the osteoid could be visualized, especially in the decortication groups. This could be suggestive of continued RAP effect. If continued force movements were applied, these samples may have had the potential for continued facilitated movement. Thus, a longer distance of movement and time points may be inquired in later studies to elucidate the later effects of these interventions.

Previous studies had suggested a decline in the RAP effect induced by Piezocision at approximately 42 days in a rodent model (Baloul et al. 2011, Dibart et al. 2013). Similar results in bone alterations in the intra-radicular region are demonstrated in these studies. After the two-

week mark, noticeable bone density changes occur and begin to re-organize by the 2-month mark. This has also been demonstrated in similar Propel studies (Alikhani et al. 2015, Teixeira et al. 2010). Literature has supported a notable and significant effect from decortication techniques in an animal model.

**Histological Findings**

Histological findings can help understand the nature of the alveolar remodeling during accelerated orthodontics. Our histological findings were also compared to the contralateral side as a control group to further develop potential contrast in changes. At day 7, all groups had a relatively similar histologic picture (Figure 17). The contralateral control sides of the jaw showed a uniform PDL space with dense alveolar bone lining the palatal aspect of the teeth. Beneath the dense cortical bone on the palatal lies a zone of less dense cancellous bone. Marrow spaces are found in this region and varied in size depending on the sample and section. Some samples had dense bone within both the furcation region and surrounding bone. Others had large marrow spaces juxtaposed to dense bone. Sites were found with disperse cancellous bone lacking a large series of Haversian systems and small dispersed marrow spaces. It could be speculated that these phenotypes may have a different response to tooth movement. The marrow spaces provide a great source of mesenchymal cells and vasculature to help remodel the bone during tooth movement (Feller et al. 2015). In a scenario of dense bone, this excellent source of remodeling factors is difficult to reach. The PDL, periosteum and intrabony cells/vasculature are relied on alone for systematically remodeling bone. This can possibly slow the rate and extent of remodeling. Possibly leading to slower or more pathological tooth movement.

The samples with larger marrow spaces may be able to facilitate cellular activity once the marrow spaces are reached. In some periodontitis studies, it has been suggested that once a marrow space is reached at crestal bone, the amount of attachment loss can accelerate leading

to a possible intrabony pocket (Soames et al. 1976). The same potential exists here, but instead a "clean" version would be occurring. The marrow space would not help propagate permanent attachment loss, but rather help modulate bone turnover. The dense surrounding bone would need to be remodeled first from other sources, especially the PDL and periosteum, in order to reach the marrow space. This theoretically can create sporadic periods of relative lag until marrow spaces are reached. Spread out, ample marrow spaces allow for continual remodeling as cells and vasculature is ample. Little remodeling is required to reach the next supply of cells and vasculature. The PDL spaces consist of a generally uniform space with dense organized ligament fibers running in varying directions. Within the dense networks, fibroblasts can be spotted relatively spread out. Vessels are also noted within this space. Larger vessels line indentations on the alveolar side of the PDL space (Figure 23). Red blood cells (RBCs) can occasionally be spotted inside the vessels. A distinct layer of bundle bone can be visualized demarcated by normal alveolar bone. Isolated osteoblasts and osteoclasts can be spotted performing routine remodeling activity. Osteocytes within lacunae in a dense network of Haversian systems are noted on the mesial and palatal of the first molar, which suggest a denser and more mature region of bone (Sodek and Mckee 2000).

### Day 7 Histology

In all groups, the day 7 histological findings remained very similar. Thus, the resulting findings to be discussed are representative of all groups. The mesial aspect of the PDL space often has a decreased width with an increased width on the distal. The compressed PDL space shows signs of hemorrhage. RBCs can be often found free floating within the PDL space (Figure 18). The lumens of the vessels are often not patent and contain many RBCs. The compression has caused blood vessels to have a constricted passage. Cells clump at these sites and limits the ability for

oxygen and other nutrient exchange. In areas of severe compression vessels may become damaged leading to excessive leakage of vascular elements into the PDL space. This spillage will likely result in the influx and activation of macrophages to clean up. The amount of blood vessel constriction and hemorrhage will be correlated to the amount of hyalinization and undermining resorption that must occur to continue tooth movement. Large multinucleated cells are found in these regions suggesting a catabolic environment has been initiated. The presence of these cells has the potential to facilitate the orchestration of the RAP effect (Frost 1983, Teixeira et al. 2010).

The PDL fibers appear to be overly dense in areas of compression and less dense in areas of tension. In areas of compression the fibers are densely packed together and have yet to be re-organized by neighboring fibroblasts. Tension areas have over stretched fibers, thus thinning out the density of collagen per histologic slice. The fibroblast orientation is very distinct in the tension region. Fibroblasts appear to be elongated and parallel to the stretched ligament fibers. Some areas appear to have torsional forces as the fibers run more parallel to the root as do the fibroblasts. In general, fibroblasts tend to be oriented parallel to the fiber orientation, which often is perpendicular to the cementum and alveolar bone (Figure 19). This ideally positions fibroblasts to migrate utilizing their leading pole along the ligament fibers and toward either cementum or bone. While moving along the fibers, these cells can help degrade and rebuild the collagenous fibers of the PDL to help re-orient the distribution of force towards homeostasis. Also, the increased access to bone and cementum aid in their relative functions at those sites, which is to breakdown and re-stablish attachment at these respective points.

In many samples, there is evidence of widening of marrow spaces (Figure 21). The marrow spaces appear to become wider within the furcation and in the bone adjacent to the bundle bone. This widening is complemented with a relative boost in cellularity. The histologic image of the widened marrow space likely indicates more marrow spaces have opened adjacent to the root on

varying vertical levels. This could give access for the marrow cells and PDL cells alike to join forces in altering the osseous architecture and facilitating the RAP effect. The trabeculation appears to create thinner peninsulas of bone in the region of the prior bundle bone. The bundle bone region in some areas has a decreased density. There is also signs of incremental lines forming at this early of a stage in the areas of decreased density. The lighter stained regions may represent new osteoid being laid down in areas of tension. The relative lightness is related to the decreased calcification of the newer immature bone. The ligament fibers appear thinner and stretched taught in these regions. The path of tension on the fibers blend in with the out stretched islands of new bone. This is a direct example of bone being built in the tension zones during orthodontic tooth movement noted in all groups.

Osteoblasts can be visualized in conjunction with osteoclasts in the remodeling zones (Figure 22 and 25). Bone formation is noted in all groups. These tensed collagen fibers are potentially great structural supports for both cell adhesion and serve as a niche for anabolic action. The mesenchymal cells and fibroblasts can utilize these firm structures to migrate to site of action. As relative tension alters, pressure changes occur allowing hydrostatic forces to affect the cells and nutrients in the environment. These trabecular patterns appear more adjacent to areas with prior cancellous bone. In regions adjacent to more cortical bone, a slightly different pattern develops. The cul-de-sacs in which the larger vessels are sitting on the outer edges of the PDL space begin to widen and become one with the PDL space. The areas with relatively large PDL spaces appear to have some protrusions of new bone forming but seems more limited than the more cancellous regions. The areas with limited PDL space and dense bone have larger resorption indentations along the bundle bone with many multinucleated cells lying within. These more cortical areas have less cellular potential adjacent to the PDL space, which could limit the ability to increase the RAP effect.

The bundle bone region does appear to be highly affected by regions of tension/pressure and cortical/cancellous in all groups. This can potentially influence the attachment apparatus and the orchestration of bone remodeling. The bundle bone in some areas is distinct but begins to become indistinguishable in some regions by day 7 depending on density (Figure 18). The denser regions with tension still retain a distinct layer of bundle bone. The denser regions with pressure seem to have a less distinguished layer of bundle bone although still evident. The osteoclastic resorption seems to be focused within the bundle bone region and has yet to expand to other regions. The vascularity and cellular source is limited to expand or moderate the remodeling and RAP effect in this region.

Regions near cancellous bone were more difficult to distinguish the bundle bone (Figure 21). The areas of tension have a soft transition from PDL fiber to osteoid to mature cancellous bone. Hence, the bundle bone may also be undergoing a maturation in these regions as well. The newly formed bone likely has collagen fibers that will take the form of Sharpey's fibers. The densifying fibers extending from the osteoid have the potential to become calcified and form the new bundle bone region. The cancellous bone with limited PDL space and pressure within the space has a mixed pattern of resorption indentations and trabecular islands. The islands of trabeculation are dense and likely not the result of newer bone formation but rather a connection of marrow spaces as result of bone remodeling. The joining of marrow spaces surrounding the test teeth can result in elaborate connections between the marrow spaces and PDL space. This increased connection reduces the surface area of bundle bone during this time point and may result in slightly greater mobility. These channels of communication also allow for osteogenic cells to travel more readily to rebuild and re-establish the proper surface areas. If osteoblastic activity is limited for the duration of tooth movement and after, attachment levels could be at risk. On

the other hand, more potential pathways for cells to travel could result in a faster and wider spread of the RAP effect.

Both in Piezocision and Propel, the site of decortication can be located at day 7(Figure 20 and 21). As the osseous injury passes through the cortical bone, the geometry of the instrumentation is distinct with an immediate zone of empty lacunae surrounding the site. Osteocytes do have extensions that reach out from the lacunae in small channels that connect to the closest lacunae and osteocytes (Sodek and Mckee 2000). It is possible that, although the lacunae have not been touched, the distal extent of the osteocytes extensions may have been damaged, and a resulting death or possible apoptosis may have occurred. In the Piezocision group, the osteocyte death appears limits to the lacunae adjacent to the injury wall (Figure 26). This limits the loss of osteocytes allowing the remaining osteocytes to respond. It has been suggested that piezosurgical osteotomies have a less damaging effect on bone tissue (Chiriac et al. 2005, Vercellotti et al. 2005), which may explain the limited damage to osteocytes seen in the Piezocision group histological samples. The osteocytes play a critical role in local bone homeostasis. Thus, a reduction of osteocytes by decortication could limit the rate of bone turnover and the rate of tooth movement.

The absence of vital osteocytes will trigger a cascade of remodeling. As the instrumentation reaches the cancellous depths, the geometry of the instrument is no longer apparent. The damage appears to elucidate a more vascular response with a localized clot forming. The clot will release a series of inflammatory cytokines and growth factors to stimulate the influx of mesenchymal and native cells (Mast and Schultz 1996). The response of bone turnover will be much greater than in the denser regions. Hence, the depth of a corticotomy is emphasized by this histological finding. The corticotomy must reach the cancellous bone to

properly initiate an effective RAP response. This also creates a pathway for communication with the devitalized cortical regions adjacent to the instrumentation.

Comparing the different measurements of tooth movement and the histological differences between groups, it appears RAP effect has yet to take on any large significance in facilitating the tooth movement at day 7. The histological findings illustrate the stage being set for the following bone turnover that will likely begin due to the osseous injury (Frost 1983). One sample in the PEMF group had a relatively large amount of bone density reduction by day 7. The nature of the pulsed fields on the alveolar bone have yet to be fully understood. The range of density changes does appear to be wide at day 7 in the PEMF group (Figure 19). Some have no apparent change at all while one appears to have the most reduction in density. It could be speculated that individuals are affected differently to the same field. This could be derived from the bone quality, genetics, and/or epigenetics. The conduction of the electromagnetic radiation is highly dependent on its penetration of the signal. Bone has a potential to transmit this energy (Diniz et al. 2002, Norton et al. 1984). Varying bone densities may respond differently to this energy input. If bone acts as an energy antenna, then the denser bone may have a resulting boost in energy influx. This boost may also react in two different ways. This influx could either transmit the energy too much or may provide a beneficial passage to the necessary cells and signals to act. On the other hand, if bone acts as an energy dampener then the passage of energy to the desired locations may be too little by the dampening or be just right. These suggestive theories would need to be further investigated to understand the exact mechanism of field therapy on tooth movement.

Individual genetic and electromagnetic responses to the electromagnetic field may also play a role. Some individuals may have a slight modification in the genes, or involved proteins in transcription, translation, and genetic ultrastructure/storage that may react differently to a

distinct applied PEMF. Applying varying PEMF as a sole variable may elucidate the specific effects on the periodontium during tooth movement. The exact mechanism of action could not be highlighted although the pattern of the bone density reduction did appear distinct from other groups. The resorption of bone followed a more radial pattern from the center of the roots. This gives the appearance of a resorption front outwards primarily from the PDL space. Other samples have more apparent tension and pressure regions. Some PEMF samples appear to be responding as a whole PDL space unit. The demineralized type 1 collagen and fibroblast rich PDL space appears to have a greater reaction to the PEMF. The PEMF applied in this study may be more attuned to the demineralized collagen spaces. It could be theorized that the wave property of this form of energy would have more of an effect on an elongated elastic fiber then a mineralized rigid fiber. The transmission could more readily transmit through the PDL fibers and effect the vasculature associated mesenchymal cells and fibroblasts within this space.

The potential for early demineralization and tooth movement was demonstrated histologically and clinically in a PEMF sample at day 7. The osseous remodeling observed in PEMF does not seem to follow that of decortication and the RAP effect. If PEMF is involved in osseous changes and accelerated tooth movement, the mechanism would not be similar to that of decortication and the RAP effect.

### Day 21 Histology

Day 21 histological findings demonstrated relatively more differences between groups. Despite more differences, certain findings remained relatively consistent amongst all groups. For example, there are regions of pressure and tension with a localized widening of PDL spaces and fusion of marrow spaces. A slight increase in the number of multinucleated cells, likely osteoclasts, are present within resorption lacunae at the edges of more cortical bone at the mesial and palatal directions. More osteoblast activity can be found at the distal sides of the molar (Figure 17). The

tension side has a greater presence of osteoid formation along the elongated fibers. Darkly stained nucleated cells line the bone sporadically, likely osteoblasts laying down premature bone. These cells will eventually become entrenched in the osteoid and form the new lacunae as bone matures. The mesial edge of the test tooth begins to enter an even denser and thinner region of bone. This could result in many potential pathological changes. Some samples had a greater reduction in bone density in this region then others, thus facilitating the movement and reducing potential pathological changes. The amount of bone remodeling had the widest range of observational variation at this time point.

The control group at day 21 appears to retain the relatively similar histologic representation as it did at day 7 (Figure 18). A slight widening of the PDL space is noted as compared to day 7. The indented regions that originally contained large vessels on the alveolar side of the PDL space has become indistinct within the PDL space. The widening and remodeling on the alveolar bone proper has widened and possibly released any potential pressure created on vessels within these regions. Some regions that demonstrate further demineralization have begun to fuse neighboring marrow spaces with the expanding PDL space. In the control group, the amount of demineralization appears to be limited. In all day 21 control samples a distinct PDL space can be identified along the entire circumference of the roots. The attachment apparatus, although altered, maintains its core components with a distinct cementum attachment and Sharpey's fibers inserted into alveolar bone proper. Localized sites of alveolar bone proper remodeling have merged the marrow spaces with the PDL space. The fibers extending from the cementum appear to divert away from the marrow spaces towards the remaining peaks of bone. In regions of large dehiscence in the alveolar bone proper, fibers appear to extend into the demineralized space. These fibers appear to weave with fibers extending from neighboring roots. This finding of extended fibers beyond alveolar bone proper is limited in the control group. This

extension of fibers suggests a possible decrease in PDL fibers directly functioning in traditional periodontal attachment. This could relate to the relative increase in mobility.

The decortication groups (Piezocision and Propel) demonstrated a potential to result in a relatively large amount of demineralization at day 21. Samples in the Propel group were able to demonstrate a nearly complete interior demineralization at day 21. Periodontal fibers still extend from cementum and intermingle with fibers from adjacent roots and eventually attach to distant bone sites. These distant bone anchorages include the mesial, distal and palatal aspects of the test molar, and occasionally the buccal aspect as well. Due to the extent of demineralization, some of the distal root fibers extend and weave with the mesial root fibers of the second molar. The demineralization can extend into the 2$^{nd}$ molar but not all the way into the 3$^{rd}$. This reflected in prior studies suggesting a potential reach of Piezocision to reach 1.5 teeth adjacent to the site of decortication (Dibart et al. 2013). Fibroblasts are highly visible amongst the reorganizing PDL fibers. As collagen turnover occurs to maintain a stable attachment apparatus, the density of mature periodontal ligament is reduced. This makes the presence of the cells more prominent, and consequently more mobile within the ligamentous space. These histological findings have been noted at similar time point in studies investigating histological changes after Piezocision (Dibart et al. 2013).

The reduction in mineralization has exposed more vascular structures, especially in the Piezocision group. The new vascularization provides greater supply for cell influx and nutrient efflux/influx. It would potentially mean that any systemic component may play a prominent role in effecting the remodeling process at this point. For example, if a systemically delivered drug designed to continue to facilitate tooth movement or help control any pathological tooth resorption existed, this would be the highly effective moment to deliver the agent. Systemic chronic inflammatory diseases, such as Diabetes and Cardiovascular disease have been correlated

with increased circulating inflammatory mediators (Kuo et al. 2008). If any of these diseases are uncontrolled, a resulting increased influx of inflammatory mediators may exacerbate the RANKL/RANK ratio within the controlled RAP effect (Zhang et al. 2017). This could lead to pathological tooth resorption or permanent loss of attachment. These factors would need to be individually studied to understand the full breadth of its potential effect on accelerated orthodontic techniques. Systemic factors and active periodontitis are both contraindications for performing acceleratory orthodontic techniques, but the underlying mechanism and direct effect is not yet fully understood.

The increased presence of blood supply can also be both a result and causative agent of the RAP effect. Since a localized increase in remodeling is performed as a result of cortical injury, the bone remodeling around the tooth exposes even more blood vessels to continue the RAP effect. If it is considered as causative agent, the localized remodeling allows more access for the vasculature to send a rapid response to the site of the cortical injury. In either scenario, the injury in conjunction with demineralization around vessels increases the remodeling in the region. Although not measured within this study, the potential for new angiogenesis is a possible component to the increased turnover. The vessels observed could be either previously present yet entrapped within the cancellous system, or they could be the result of angiogenesis within the region. The RAP effect does have the potential to induce angiogenesis to bring in the required components to facilitate repair. Angiogenesis often stems from present vasculature. Since the site of cortical bone injury must rely on blood supply from the cancellous bone, periosteum, and the PDL space to establish the RAP effect and repair the injury. Considering the density of the vessels within the PDL space, it is possible that the vessels needed to stimulate the RAP response are derived mostly from the PDL. The young vessels may be the reason for increased cellular presence. The vessels also appear to be less compressed in the wider PDL complex. The newly remodeling

fibers are less dense and allow for easier cell permeability. Cells can reach site of needed response quicker and propagate the healing response faster.

Much of the cellular activity does appear to be in the alveolar side of the PDL space. With the increased presence of bone resorptive activity, the bundle bone is actively changing. The density of the bundle bone region is decreased resulting in less ligament anchorage in bone. This is a critical portion of the periodontal attachment apparatus. The body must quickly be able to reorganize the fibers to establish adequate attachment. This requires an orchestration of collagen degradation and re-organization amongst the array of cellular activity required to move the tooth. It is conceivable that the body is constantly attempting to maintain a homeostatic relationship of ligament fibers within the periodontal space. This could be analogous to a biologic width. Instead of a connective tissue and junctional epithelium this biologic dimension is within the PDL space between the bundle bone and the cementum. The resulting remodeling from changes in pressure and tension could be an attempt to maintain the horizontal PDL biologic width dimension. During tooth movement, this biologic width has the ability to undergo substantial remodeling to prevent over compression or over tension of fibers. The increased extracellular activity to remodel would need to be on the alveolar bone side as we do not have an efficient means to quickly remodel cementum and dentin. Like the crestal alveolar bone, having an innate increased nutrient canal presence may be a critical factor in establishing remodeling at the alveolar side.

A denser bundle bone may inhibit the cellular presence on the alveolar side during remodeling. As the PDL space is reduced in pressure regions, the active cells become closer to cementum and dentin. The earlier catabolic steps of remodeling may abnormally effect root components which can not sufficiently anabolically rebuild, possibly resulting in root resorption and/or ankyloses (Figure 22). Thus, the apparent increase in active cells dispersed within a relatively more demineralized region helps prevent pathological components of an “invasion of

biologic width" within the PDL space during tooth movement. It is known that upon flap reflection crestal bone undergoes a certain level of remodeling that involves a stage that resembles the RAP phenomenon (Wood et al. 1972). At this time point the biologic width is temporarily altered but will re-establish. This intermediate stage is very similar to what is observed at the mid root at certain day 21samples. A transient increase in the biologic dimension of the PDL is observed, but potential for rebuilding exists as long as the factors causing the change are transient.

As these fibroblasts are actively remodeling the attachment apparatus, the teeth are looser. This was confirmed as several day 21 samples in the Piezocision group were avulsed during sectioning of the sample. The teeth had a loosened attachment and were highly mobile. The histological appearance if these samples and similar samples reflected a loose connective tissue zone extending far from the root. The fibroblasts are actively secreting collagenase to re-organize fibers and osteoclasts have removed bundle bone. The fibers extend longer into wide nutrient canals and sometimes into the neighboring root ligaments.

A short taut fiber during normal conditions firmly anchors teeth. As the density of fibers reduces and fibers are longer, the elastic nature of collagen allows for greater movement until the fibers are fully taut. This looser wider PDL space at day 21 can translate into highly mobile teeth. The mobility is not a direct concern. Under uncontrolled occlusion, bacterial inflammation, and certain systemic complications, this remodeling may be difficult to rebuild. In those scenarios, accelerated orthodontics should not be performed. If these components are controlled, the mobility is then simply a result of transient changes in the periodontium and will resolve as the rebuilding of the attachment apparatus occurs. The same cell rich fibroblast regions involved in reducing the density of fibers will be responsible for rebuilding the dense ligament network once the orthodontic movement is complete and the RAP effect ends.

The signal based osteoclastic activity appears high at day 21. The osteoclast activity is in close relation to the neighboring fibroblast activity at this point. Signals may be translating bi-directionally in order to orchestrate the reduction in density of bone and fibers to allow for tooth movement. The addition of cortical perforations can change the number of cells present to facilitate this process. This is suggested by the amount of remodeling seen in the several day 21 samples in the Piezocision and Propel groups.

Although the PEMF group often resembled the control group at day 21, one sample demonstrated a large radius of remodeling around the test roots. This sample had remodeling almost entirely isolated to the involved teeth. The samples in the decortication groups that had a large amount of demineralization, the effects were more diffuse. The PEMF sample had a radial pattern of demineralization from the roots, giving the appearance of an evenly widened PDL space. The electromagnetic field is pulsed, and the pattern of pulsing is variant and may appear as a pulsing pattern within the tissue. The inductive mat produces low frequency waveform electromagnetic energy (Shupak et al. 2003). The moments the frequency fluctuates could result in intermittent moments of remodeling. The amount of time it would take for remodeling to occur would be too long to be directly affected by the relatively quick variants in energy production.

It is more likely that the cumulative effect of the energy would develop the effect rather than intermittent moments of osteoclastic activity. Most studied uses of PEMF focus on its potential for osteoblastic activity induction and acceleration (Diniz et al. 2002, Esther 2010, Zhang et al. 2017, Satake 1990, Yang 2015. For tooth movement, osteoclastic activity must also be altered to accelerate tooth movement. Some evidence has suggested a down regulation in RANKL dependent induction of osteoclastic activity created by PEMF (Zhang et al. 2017). But to sufficiently remodel, a period of increased osteoclastic activity is required. It may be possible that the PEMF is causing a reduction in osteoclastic activity and an increase in osteoblastic maturation.

This would explain the lack of remodeling and the nearly pristine density of bone noted in most of the PEMF samples. Thus, negating the acceleratory healing benefits of the PEMF and having limited results in bone demineralization and accelerated tooth movement. Due to the intermittent nature of the PEMF, it is also possible that the continuous force applied by the orthodontic appliance is producing an osteoclastic effect that is masking the osteoblastic potential of the PEMF in several samples showing increased demineralization.

The original purpose of the PEMF inductive mat was designed for dental bone healing, not dental bone demineralization. This was supported in this study with the relatively limited results in demineralization. The ability to induce osteoblastic ability may even possibly hinder proper osteoclastic activity and throttle the potential for fast tooth movement, as demonstrated by the relatively limited rate of tooth movement. The sample with relatively large amounts of demineralization may have been due to the orthodontic appliance overriding the effects of the PEMF. This specific sample may have had an ideally placed orthodontic appliance supplying maximum forces to initiate the demineralization typically involved in heavy orthodontic forces. It may also be the result of PEMF interplay with genetics. The genetic variability of PEMF on individuals has not been fully investigated and may be play a role in its effectiveness. Although upregulation of osteoblastic activity has been studied, histological studies do not find an increase in the number of osteoblasts. They suggest the anabolic potential is derived from increased osteoblast quality but not the quantity. Our histological findings could not show any increase in the number of osteoblasts present. Our findings may continue to support prior research suggesting PEMF's osteoblastic potential and limiting abilities on osteoclasts.

### Day 49 Histology

The day 49 samples began to show signs of remineralization. At this time point, much of the orthodontic movement is completed for all groups due to the amount of force applied. Some

groups may have finished prior to this time point. In theory, the earlier the completion of the orthodontic movement the more rebuilding or remineralization could have occurred. The control group continued to maintain a relatively similar distribution of mineralization as it did in prior time points. A couple of the control group samples that did not reach the full length of tooth movement possible had a histological appearance of bone similar to day 7. The architecture of the bone resembles the cancellous nature of the control side with a slight increase in demineralization and larger PDL/marrow spaces. The PEMF group had a similar pattern of osseous changes in samples that did not complete movement. The samples that did not finish at day 49 in the decortication groups had signs of greater demineralization suggesting a potential for continued movement. The efficiency of the appliance in these samples may have limited the full movement of the tooth, and/or the osteoblastic remineralization may be rebuilding the osseous architecture. The RAP effect at this point may be beginning its last stage of healing and slowly coming to an end. This could signal the nearing end of accelerated potential of the decortication.

The samples that did complete the movement had similar bone patterns in all groups except for two in the PEMF group. In these samples bone patterns around the midroot region resembled more the native cancellous architecture noted at day 7. The PEMF group at no time point in any simple resulted in significant enough demineralization to potentially alter the ultrastructure of the cancellous bone. The density and architecture of attachment most resembles the tooth's initial presentation at day 7. The periodontal attachment has the most potential to return to baseline. The cell quantity does not appear overabundant at any time point. This may be due to the osteoblastic effects of the PEMF. The number of osteoblasts were not directly measured, but the nucleated cells lining the alveolus does not appear to be distinctly abundant. The PEMF may be improving the osteoblastic efficiency to maintain the alveolar architecture, and in turn maintaining the bundle bone/periodontal attachment. The lack of significant

demineralization could potentially limit the movement rate. Although the density of bone could also result in more pathological orthodontic related root resorption, the ability to improve osteoblastic activity may limit catabolic pathology.

The decortication groups had a unique reorganization of the surrounding alveolar bone at day 49. The cancellous bone has a very organized pattern. Thin streaks of alveolar bone are interlaced with thin elongated marrow space (Figure 24). The bone and marrow spaces are interlaced in a stripe-like pattern. The elongation is parallel towards the long axis of the tooth. The teeth often are tipped mesially at the end of complete movement. As mentioned, the limitation of the bone found mesial to the test molar in conjunction with the orthodontic appliance, produce a slight tilt in the molar towards the end of movement. The fibers extending from the cementum are producing tension against the long axis of the tooth during this tilting to produce resistance to the avulsion forces being applied. As the tooth eventually hits a point beyond the potential effects of the decortication induced RAP, the dense mesial bone produces a point of resistance. A fulcrum point is created, and the tooth begins to rotate around the point of resistance. This results in tilting creating a new force vector being applied outwards or coronally on the tooth. The new direction of force causes tension in apical fibers. These fibers are loci of osteoblastic rebuilding. Considering the relative amount of demineralization and increased cellular activity, these fibers are being remineralized relative to the tensed fibers. The newly formed bone resembles an osteoid with limited to no osteocyte presence. The amount of distinct bundle bone appears limited and immature. As remineralization continues, the distinction between alveolar bone and bundle bone may become more distinct.

Having the reformation of bundle bone after having an episode of near complete bundle bone breakdown is a relieving sign to see. In the lack of a continuous stimuli of inflammation, such as decortication, tooth movement, and bacteria, the attachment apparatus will attempt to re-

establish. The biological width of the PDL space is attempted to reform in order provide a homeostatic relationship in tension of fibers. As maturation continues, the likely effect on the pattern of osteoid and marrow spaces will continue to have morphological changes. The initial goal is to re-establish attachment, which appears to have been accomplished quickly after stimuli removal. After the security of the tooth's attachment is done, the alveolus can now begin to remodel to optimize the distribution of force and fiber tension. The current architecture of the alveolus does appear to have developed without the need for consideration of the occlusion. In the tooth's final position, there is no occlusion. If the tooth had been mobilized to an area of occlusion, a different architecture may have formed in order to both compensate for the forces developed by orthodontics and occlusion. The axial elongated thin strips of bone and marrow spaces provides limited architectural support in the axial direction but does allow for some flexure strength. This reflects the lack of occlusal forces applied after mesialization and the continuous nonaxial pulling force applied by the appliance.

A relatively dense region of fibroblasts can still be noted towards the alveolar side of the PDL. Less is apparent than the day 21 samples. Densely stained cells can be found in a linear fashion along the immature bundle bone. These are likely osteoblasts continuing the maturation of the bundle bone to further solidify the attachment. Sparse multinucleated cells can now be found. The number of macrophages and osteoclasts needed at this time point are reduced. This shifts the cellular content and likely the cytokine ratios (Baloul et al. 2011).

The histological evidence demonstrates a greater potential for movement in decortication groups, as compared to control and PEMF. Piezocision was shown to significantly reduce the bone density surrounding the roots of the first molar allowing for easier tooth movement. The effect requires more than a week to begin. Within one week, not enough time has passed to allow for cells to ramp a complete response to the cortical injury. As cortical bone

is turned over to allow for more vessels and cells to respond to the injury, the response to the orthodontic tooth movement increases as well. The same angiogenesis and cytokines begin to affect the machinery involved in tooth movement. The RAP effect is visualized histologically with a decrease in mineralization and increase in cellular orchestration. At 21 days, this can produce a potentially scarce amount of periodontal attachment. The bundle bone absence and PDL fiber density reduction lay a potentially fragile amount of attachment for the test tooth. The effect appears to spread to the neighboring non-moved tooth as well. The Piezocision group had a greater potential for demineralization, PDL fiber density reduction, and increased cellularity than Propel. But the need for attachment drives the healing response to re-establish an attachment at the end of tooth movement. Remineralization occurs and the PDL fibers begin to return to a baseline density. Cellular content begins to also revert to normal. Overall, movement is temporarily easier through a transient demineralized region with reduced, restricting attachment. Although PEMF may also have an effect on orthodontic tooth movement, it may not be able to facilitate faster tooth movement. The PEMF appears to retain baseline bone quality with limited catabolic activity. This may be due to the osteoblastic inductivity of PEMF. There is limited evidence to suggest that PEMF has the ability to produce demineralization or upregulate osteoclastic activity. The effects do not stop tooth movement but does appear to maintain baseline bone architecture. Even after completed tooth movement by day 49, the bone appears more similar to baseline then does the control group. A more distinct bundle bone and attachment apparatus is apparent. It may be possible that the reduction in catabolic activity may help prevent pathological resorption during tooth movement. It may be conceivable that higher forces with PEMF could be used to achieve faster tooth movement with protection against pathological tooth changes associated with excessive forces. This has not been directly studied

and may be a potential path of future research. Cytokine measurements should also be investigated in future research to further the understanding of PEMF and tooth movement.

**Decortication Groups**

Despite both Piezocision and Propel being a form of decortication, the Piezocision group was able to radiographically and histologically reduce the bone density more initially and maintain a higher mean of radiographic density reduction. One factor that could explain the difference could be the relative size of the decortication. Piezocision decortications may be larger than Propel decortications. Although a relative correlation has not been established between injury size and the amount of RAP, more injury could require a greater inflammatory influx and a larger/longer RAP effect. In larger animals and humans, it is recommended to provide more than one Propel micro-osteoperforation to accelerate tooth movement. This increases the amount of decortication and may resemble the injury size of Piezocision. The modified piezosurgical tip used in an animal model is much smaller than the suggested inserts on humans. The size of the injury more closely resembles the diameter of the Propel instrument. The relative sizes of decortication were similar in both groups with the potential of Piezocision having a slightly larger, no greater than 0.5mm, injury.

Another explanation could be the osteogenic potential delivered by piezoelectric instruments. It can be speculated that the electromagnetic properties may have been transmitted to the bone via the piezosurgical instrument. The dissipation of that energy through the site of injury could have facilitated the greater presence of osteogenic cells beyond a simple decortication (Vercellotti et al. 2005). The extra signaling factor of electromagnetic radiation in the frequency range of RAP related cells could have caused an increase in bone remodeling and

bone density reduction. However, no direct correlation of this relationship could be derived from this study.

## Clinical Implications

Although the rodent molar mesialization model does have its limitations, it serves as an excellent early investigation model for in vivo histologic observation of tooth movement. Results investigated in this model still should await further validation from larger animal model studies. The ability to achieve histologic representation is key in understanding the fundamental effects of minimally invasive accelerated orthodontics on the periodontium. This information allows clinicians to better understand the nature and efficiency of the procedures performed. Knowing the potential of the demineralization of the RAP inducing procedures, special care should be done to preventing detrimental attachment loss or avulsion after a couple weeks of decortication. Loose PDL fibers are much of the attachment in this phase resulting in significant mobility. Knowing that remineralization will occur, allows us to safely move through the transient osteopenia phase with confidence that attachment will not be harmed. Due to the amount of demineralization, the orthodontist may have to maintain the appliance as a splint or utilize a temporary fixed retainer in order to allow for proper bone maturation.

Another component of Piezocision is the ability to graft. Often a thin biotype would need to be converted into a thick biotype. Thin soft and hard tissue can result in recession depending on the direction of movement, frenum attachments, brushing habits, and plaque level (Yared et al. 2006, Joss-Vassalli et al. 2010). When significant buccal movements are being performed on a thin tissue biotype, recession must be considered a possibility. A thorough periodontal examination in conjunction with advanced dental imaging can be used to better predict potential recession. PAOO has been suggested to be successful in increasing the tissue biotype, even during buccal advancement (Wilcko et al. 2015). This is because this technique allows for sufficient access

to graft by creating a flap. Piezocision creates full thickness vertical incisions which can be connected via an internal flap. The created space allows for the passage of graft in a minimally invasive fashion. No reflection of papilla or marginal tissue is required to be able to pass hard and soft tissue grafting into the tunneled space. The decortication also allows for an adequate blood supply, especially as the Rap effect begins to take place at day 21. The increase in cellularity and potential pathway for angiogenesis can potentially boost the healing of the graft. This technique retains the ability of the graft while reducing the amount of surgery, which improves patient and orthodontist compliance. PEMF and Propel both do not give sufficient access to be able to graft the buccal tissues of relatively thin sites.

If studies continue to demonstrate the ability for PEMF to control catabolic activity and upregulate anabolic remodeling, then it could be used in incidences when pathological root resorption or ankyloses is anticipated. It could also be used in later stages of orthodontic movement, especially in cases where decortications were used. The amount of demineralization is impressive after decortication. Towards the completion of tooth movement, PEMF could be utilized for re-establishing the mineralization and maturity of bone. Another future study could observe the amount of time needed to retain orthodontic appliances for bone maturation in controls compared to PEMF used after tooth movement completion.

**Conclusions**

- Minimally invasive decortication techniques produce the RAP effect to facilitate tooth movement.
- Piezocision may produce greater reduction in bone density to facilitate faster tooth movement.
- The rodent orthodontic model is advantageous for observing radiographic and histological changes resulting from accelerated orthodontic techniques.

- Pulsed electromagnetic field therapy may enhance osteoblastic activity and does not facilitate the RAP effect of the alveolar bone.

# References


1. Abdallah, M. N., & Flores-Mir, C. (2014). Are interventions for accelerating orthodontic tooth movement effective?. Evidence-based dentistry, 15(4), 116-117.

2. Abu-Hussein, M., Watted, N., Hegedűs, V., & Borbély, P. (2015). Corticotomy in the Modern Orthodontics. Journal of Dental and Medical Sciences (IOSR-JDMS) Volume 14, Issue 11 Ver. III, PP 68-80.

3. Addy, M., Shaw, W. C., Hansford, P., & Hopkins, M. (1982). The effect of orthodontic appliances on the distribution of Candida and plaque in adolescents. Journal of Orthodontics, 9(3), 158-163.

4. Ahn, A. C., & Grodzinsky, A. J. (2009). Relevance of collagen piezoelectricity to "Wolff's Law": a critical review. *Medical engineering & physics*, *31*(7), 733-741.

5. Ainamo, J. (1972). Relationship between malalignment of the teeth and periodontal disease. *European Journal of Oral Sciences*, *80*(2), 104-110.

6. Åkesson, L., Håkansson, J., & Rohlin, M. (1992). Comparison of panoramic and intraoral radiography and pocket probing for the measurement of the marginal bone level. *Journal of clinical periodontology*, *19*(5), 326-332.

7. Alikhani, M., Alansari, S., Sangsuwon, C., Alikhani, M., Chou, M. Y., Alyami, B., Teixeira, C. C. (2015). Micro-osteoperforations: minimally invasive accelerated tooth movement. In *Seminars in Orthodontics* (Vol. 21, No. 3, pp. 162-169).

8. Alikhani, M., Raptis, M., Zoldan, B., Sangsuwon, C., Lee, Y. B., Alyami, B., & Teixeira, C. (2013). Effect of micro-osteoperforations on the rate of tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics, 144(5), 639-648.

9. Anderson, J. C., & Eriksson, C. (1970). Piezoelectric properties of dry and wet bone. *Nature*, *227*(5257), 491-492.

10. Arias, O. R., & Marquez-Orozco, M. C. (2006). Aspirin, acetaminophen, and ibuprofen: their effects on orthodontic tooth movement. *American Journal of Orthodontics and Dentofacial Orthopedics*, *130*(3), 364-370.

11. Baloul, S. S., Gerstenfeld, L. C., Morgan, E. F., Carvalho, R. S., Van Dyke, T. E., & Kantarci, A. (2011). Mechanism of action and morphologic changes in the alveolar bone in response

to selective alveolar decortication–facilitated tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics, 139(4), S83-S101.

12. Bambini, F., Santarelli, A., Putignano, A., Procaccini, M., Orsini, G., Memè, L., & Lo, M. L. (2017). Use of supercharged cover screw as static magnetic field generator for bone healing, 1st part: in vitro enhancement of osteoblast-like cell differentiation. Journal of biological regulators and homeostatic agents, 31(1), 215.

13. Barak, S., Neuman, M., Iezzi, G., Piattelli, A., Perrotti, V., & Gabet, Y. (2016). A new device for improving dental implants anchorage: a histological and micro-computed tomography study in the rabbit. Clinical oral implants research, 27(8), 935-942.

14. Bemard Gantes, E. R. (1990). Effects on the Periodontium Following Corticotomy-Facilitated Orthodontics: Case Reports. Journal of Periodontology, 61 (4), 234-238.

15. Boyd, R. L., & Baumrind, S. (1992). Periodontal considerations in the use of bonds or bands on molars in adolescents and adults. The Angle Orthodontist, 62(2), 117-126.

16. Burger EH, Klein-Nulen J. (1999). Responses of bone cells to biomechanical forces in vitro. Adv Dent Res;13:93–8.

17. Zhu, J., Zhang, X., Wang, C., Peng, X., & Zhang, X. (2008). Different magnitudes of tensile strain induce human osteoblasts differentiation associated with the activation of ERK1/2 phosphorylation. International journal of molecular sciences, 9(12), 2322-2332.

18. Charavet, C., Lecloux, G., Bruwier, A., Rompen, E., Maes, N., Limme, M., & Lambert, F. (2016). Localized Piezoelectric Alveolar Decortication for Orthodontic Treatment in Adults A Randomized Controlled Trial. Journal of dental research.

19. Chen, Y. J., Jeng, J. H., Chang, H. H., Huang, M. Y., Tsai, F. F., & Jane Yao, C. C. (2013). Differential regulation of collagen, lysyl oxidase and MMP-2 in human periodontal ligament cells by low-and high-level mechanical stretching. *Journal of periodontal research*, *48*(4), 466-474.

20. Chen, Y., Mohammed, A., Oubaidin, M., Evans, C. A., Zhou, X., Luan, X., & Atsawasuwan, P. (2015). Cyclic stretch and compression forces alter microRNA-29 expression of human periodontal ligament cells. *Gene*, *566*(1), 13-17.

21. Chiriac G, Herten M, Schwarz F, et al. (2005). Autogenous bone chips: influence of a new piezoelectric device (Piezosurgery) on chip morphology, cell viability and differentiation. J Clin Periodontol;32:994Y999.

22. Choo, H., Heo, H. A., Yoon, H. J., Chung, K. R., & Kim, S. H. (2011). Treatment outcome analysis of speedy surgical orthodontics for adults with maxillary protrusion. *American Journal of Orthodontics and Dentofacial Orthopedics*, *140*(6), e251-e262.

23. Cowin SC, Moss-Salentijn L, Moss ML. (1991). Candidates for the mechanosensory system in bone. J Biomech Eng;113(2):191–7.

24. Deguchi, T., Seiryu, M., Daimaruya, T., Garetto, L. P., Takano-Yamamoto, T., & Roberts, W. E. (2014). Decreased alveolar bone turnover is related to the occurrence of root resorption during experimental tooth movement in dogs. The Angle Orthodontist, 85(3), 386-393.

25. Dibart, S., Sebaoun, J. D., & Surmenian, J. (2011). Piezocision: Minimally Invasive Periodontally Accelerated Orthodontic Tooth Movement Procedure. Practical Osseous Surgery in Periodontics and Implant Dentistry, 195.

26. Dibart, S., Yee, C., Surmenian, J., Sebaoun, J. D., Baloul, S., Goguet-Surmenian, E., & Kantarci, A. (2013). Tissue response during Piezocision-assisted tooth movement: a histological study in rats. The European Journal of Orthodontics, cjt079.

27. Diniz, P., Shomura, K., Soejima, K., & Ito, G. (2002). Effects of pulsed electromagnetic field (PEMF) stimulation on bone tissue like formation are dependent on the maturation stages of the osteoblasts. Bioelectromagnetics, 23(5), 398-405.

28. Dogru, M., Akpolat, V., Dogru, A. G., Karadede, B., Akkurt, A., & Karadede, M. I. (2014). Examination of extremely low frequency electromagnetic fields on orthodontic tooth movement in rats. Biotechnology & Biotechnological Equipment, 28(1), 118-122.

29. Donahue, H. J. (2000). “Gap junctions and biophysical regulation of bone cell differentiation,” Bone, vol. 26, no. 5, pp. 417–422.

30. Esther Lau, S. A.-D. (2010). Effect of low-magnitude, high-frequency vibration on osteocytes in the regulation of osteoclasts. Bone, 46, 1508-1515.

31. Feller, L., Khammissa, R. A. G., Schechter, I., Thomadakis, G., Fourie, J., & Lemmer, J. (2015). Biological events in periodontal ligament and alveolar bone associated with application of orthodontic forces. *The Scientific World Journal*.

32. Frost, H. M. (1983). The Regional Acceleratory Phenomenon, A Review. Henry Ford Hospital Medical Journal, 31 (1), 3-9.

33. Gonzales, C., Hotokezaka, H., Yoshimatsu, M., Yozgatian, J. H., Darendeliler, M. A., & Yoshida, N. (2008). Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. *The Angle orthodontist*, *78*(3), 502-509.

34. Grimard, B. A., Hoidal, M. J., Mills, M. P., Mellonig, J. T., Nummikoski, P. V., & Mealey, B. L. (2009). Comparison of clinical, periapical radiograph, and cone-beam volume tomography measurement techniques for assessing bone level changes following regenerative periodontal therapy. *Journal of periodontology*, *80*(1), 48-55.

35. Halperin C, Mutchnik S, Agronin A, et al. (2004). Piezoelectric effect in human bones studied in nanometer scale. Nano Lett;4(7):1253–6.

36. Hannemann, P. F. W., Mommers, E. H. H., Schots, J. P. M., Brink, P. R. G., & Poeze, M. (2014). The effects of low-intensity pulsed ultrasound and pulsed electromagnetic fields bone growth stimulation in acute fractures: a systematic review and meta-analysis of randomized controlled trials. Archives of orthopaedic and trauma surgery, 134(8), 1093-1106.

37. Horton JE, Tarpley TM Jr, Jacoway JR. (1981). Clinical applications of ultrasonic instrumentation in the surgical removal of bone. Oral Surgery Oral Medicine Oral Pathology Oral Radiology, 51, 236–242.

38. Huang, H., R. D. Kamm, and R. T. Lee, (2004). "Cell mechanics and mechanotransduction: pathways, probes, and physiology," The American Journal of Physiology—Cell Physiology, vol. 287, no. 1, pp. C1–C11.

39. Ibrahim, A. Y., Gudhimella, S., Pandruvada, S. N., & Huja, S. S. (2017). Resolving differences between animal models for expedited orthodontic tooth movement. *Orthodontics & Craniofacial Research*, *20*(S1), 72-76.

40. Sebaoun, A. K. (2008). Modeling of Trabecular Bone and Lamina Dura Following Selective Alveolar Decortication in Rats. Journal of Periodontology, 79 (9), 1679-1688.

41. Joss-Vassalli, I., Grebenstein, C., Topouzelis, N., Sculean, A., & Katsaros, C. (2010). Orthodontic therapy and gingival recession: a systematic review. Orthodontics & Craniofacial Research, 13(3), 127-141.

42. Kim, K. T. (1990). Biochemical study of human periodontal ligament: preparation of cell attachment materials induced by pulsed electromagnetic fields. The Bulletin of the Kanagawa Dental College: BKDC/KDS, 18(2), 89-98.

43. Krishnan, V., & Davidovitch, Z. E. (2006). Cellular, molecular, and tissue-level reactions to orthodontic force. *American Journal of Orthodontics and Dentofacial Orthopedics*, *129*(4), 469-e1.

44. Kuo, L. C., Polson, A. M., & Kang, T. (2008). Associations between periodontal diseases and systemic diseases: a review of the inter-relationships and interactions with diabetes,

respiratory diseases, cardiovascular diseases and osteoporosis. *Public health*, *122*(4), 417-433.

45. Makki, L., Ferguson, D. J., Wilcko, M. T., Wilcko, W. M., Bjerklin, K., Stapelberg, R., & Al-Mulla, A. (2014). Mandibular irregularity index stability following alveolar corticotomy and grafting: A 10-year preliminary study. *The Angle Orthodontist*, *85*(5), 743-749.

46. Marino, A. A., & Gross, B. D. (1989). Piezoelectricity in cementum, dentine and bone. *Archives of oral biology*, *34*(7), 507-509.

47. Marzec E, Kubisz L, Jaroszyk F. (1996). Dielectric studies of proton transport in airdried fully calcified and decalcified bone. Int J Biol Macromol;18(1–2):27– 31.

48. Masella, R.S. and M. Meister. (2006). "Current concepts in the biology of orthodontic tooth movement," American Journal of Orthodontics and Dentofacial Orthopedics, vol. 129, no. 4, pp. 458–468.

49. Mast, B. A., & Schultz, G. S. (1996). Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Repair and Regeneration*, *4*(4), 411-420.

50. Melo, A. C. E. D. O., Carneiro, L. O. T., Pontes, L. F., Cecim, R. L., Mattos, J. N. R. D., & Normando, D. (2013). Factors related to orthodontic treatment time in adult patients. *Dental press journal of orthodontics*, *18*(5), 59-63.

51. Miyagawa, A., Chiba, M., Hayashi, H., & Igarashi, K. (2009). Compressive force induces VEGF production in periodontal tissues. *Journal of Dental Research*, *88*(8), 752-756.

52. Mouraret, S., Houschyar, K. S., Hunter, D. J., Smith, A. A., Jew, O. S., Girod, S., & Helms, J. A. (2014). Cell viability after osteotomy and bone harvesting: comparison of piezoelectric surgery and conventional bur. *International journal of oral and maxillofacial surgery*, *43*(8), 966-971.

53. Murphy, C. A., Chandhoke, T., Kalajzic, Z., Flynn, R., Utreja, A., Wadhwa, S., & Uribe, F. (2014). Effect of corticision and different force magnitudes on orthodontic tooth movement in a rat model. *American Journal of Orthodontics and Dentofacial Orthopedics*, *146*(1), 55-66.

54. Murshid, S. A. (2017). The role of osteocytes during experimental orthodontic tooth movement: A review. Archives of oral biology, 73, 25-33.

55. Netto, T. G., & Zimmerman, R. L. (1975). Effect of water on piezoelectricity in bone and collagen. *Biophysical journal*, *15*(6), 573-576.

56. Norton, L. A., Hanley, K. J., & Turkewicz, J. (1984). Bioelectric Perturbations of Bone: Research Directions and Clinical Applications. The Angle Orthodontist, 54(1), 73-87.

57. Park, Y. G., Kang, S. G., & Kim, S. J. (2006). Accelerated tooth movement by corticision as an osseous orthodontic paradigm. *Kinki Tokai Kyosei Shika Gakkai Gakujyutsu Taikai, Sokai*, *48*(6), 34.

58. Ramani-Mohan, R. K., Schwedhelm, I., Finne-Wistrand, A., Krug, M., Schwarz, T., Jakob, F., ... & Hansmann, J. (2017). Deformation strain is the main physical driver for skeletal precursors to undergo osteogenesis in earlier stages of osteogenic cell maturation. *Journal of tissue engineering and regenerative medicine*.

59. Ren, L., Yang, P., Wang, Z., Zhang, J., Ding, C., & Shang, P. (2015). Biomechanical and biophysical environment of bone from the macroscopic to the pericellular and molecular level. *Journal of the mechanical behavior of biomedical materials*, *50*, 104-122.

60. Rosa N, Simoes R, Magalhães FD, Marques AT. (2015). From mechanical stimulus to bone formation: A review. Medical engineering & physics 37(8):719-728.

61. Rubin, C. T., Donahue, H. J., Rubin, J. E., & McLeod, K. J. (1993). Optimization of electric field parameters for the control of bone remodeling: exploitation of an indigenous mechanism for the prevention of osteopenia. Journal of Bone and Mineral Research, 8(S2), S573-S581.

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

63. Satake, T. (1990). Effect of pulsed electromagnetic fields (PEMF) on osteoblast-like cells. Alterations of intracellular Ca2+. Kanagawa shigaku. The Journal of the Kanagawa Odontological Society, 24(4), 692-701.

64. Sato, T., J. Iida, and S. Kurihara. (1984). A histological study of the periodontal tissue changes during molar depression in rats. J Jpn Orthod Soc. 43:361–372.

65. Shenava, S., Nayak, U. K., Bhaskar, V., & Nayak, A. (2014). Accelerated Orthodontics–A Review.

66. Shupak, N. M., Prato, F. S., & Thomas, A. W. (2003). Therapeutic uses of pulsed magnetic-field exposure: a review. *URSI Radio Science Bulletin*, *76*(4), 9-32.

67. Skidmore, K. J., Brook, K. J., Thomson, W. M., & Harding, W. J. (2006). Factors influencing treatment time in orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopedics*, *129*(2), 230-238.

68. Skomro, P., Lietz-Kijak, D., Kijak, E., Bogdziewicz-Wałęsa, O., & Opalko, K. (2012). The change of electric potentials in the oral cavity after application of extremely low frequency pulsed magnetic field. Advances in Hygiene & Experimental Medicine/Postepy Higieny i Medycyny Doswiadczalnej, 66.

69. Soames, J. V., Entwisle, D. N., & Davies, R. M. (1976). The progression of gingivitis to periodontitis in the beagle dog: a histological and morphometric investigation. *Journal of periodontology*, *47*(8), 435-439.

70. Sodek, J., & Mckee, M. D. (2000). Molecular and cellular biology of alveolar bone. *Periodontology 2000*, *24*(1), 99-126.

71. Stark, T. M., & Sinclair, P. M. (1987). Effect of pulsed electromagnetic fields on orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics, 91(2), 91-104.

72. Stroe, M. C., Crolet, J. M., & Racila, M. (2013). Mechanotransduction in cortical bone and the role of piezoelectricity: a numerical approach. *Computer methods in biomechanics and biomedical engineering*, *16*(2), 119-129.

73. Teixeira, C. C., Khoo, E., Tran, J., Chartres, I., Liu, Y., Thant, L. M., & Alikhani, M. (2010). Cytokine expression and accelerated tooth movement. *Journal of dental research*, *89*(10), 1135-1141.

74. Thomas, M., Akula, U., Ealla, K. K., & Gajjada, N. (2017). Piezosurgery: A boon for modern periodontics. *Journal of International Society of Preventive & Community Dentistry*, *7*(1), 1.

75. Tomaso Vercellotti, M. D. (2005). Osseous Response Following Resective Therapy with Piezosurgery. The International Journal of Periodontics & Restorative Dentistry, 25 (6), 543-549.

76. Uribe, F., Padala, S., Allareddy, V., & Nanda, R. (2014). Patients', parents', and orthodontists' perceptions of the need for and costs of additional procedures to reduce treatment time. *American Journal of Orthodontics and Dentofacial Orthopedics*, *145*(4), S65-S73.

77. Varela, M., & García-Camba, J. E. (1995). Impact of orthodontics on the psychologic profile of adult patients: a prospective study. *American Journal of Orthodontics and Dentofacial Orthopedics*, *108*(2), 142-148.

78. Vercellotti, T., Nevins, M. L., Kim, D. M., Nevins, M., Wada, K., Schenk, R. K., & Fiorellini, J. P. (2005). Osseous response following resective therapy with piezosurgery. International Journal of Periodontics & Restorative Dentistry, 25(6).

79. Wang, X., Shen, X., Li, X., & Agrawal, C. M. (2002). Age-related changes in the collagen network and toughness of bone. *Bone*, *31*(1), 1-7.

80. Weyand, C. M., Yang, Z., & Goronzy, J. J. (2014). T cell aging in rheumatoid arthritis. *Current opinion in rheumatology*, *26*(1), 93.

81. Wilcko, W. W. (2013). Accelerating tooth movement: The case for corticotomy-induced orthodontics. American Journal of Orthodontics and Dentofacial Orthopedics, 144 (1), 4-12.

82. Wilcko WM, Ferguson DJ, Bouquot JE, et al. (2003). Rapid orthodontic decrowding with alveolar augmentation: case report. World Journal of Orthodontics, 4, 197-505.

83. Wilcko, M. T., Ferguson, D. J., Makki, L., & Wilcko, W. M. (2015). Keratinized gingiva height increases after alveolar corticotomy and augmentation bone grafting. *Journal of periodontology*, *86*(10), 1107-1115.

84. Wilcko MT, Wilcko WM et al. (2009). Accelerated osteogenic orthodontics technique. J Oral Maxillofac Surg; 67:2149.

85. Will, L. A. (2016). Orthodontic Tooth Movement: A Historic Prospective. In *Tooth Movement* (Vol. 18, pp. 46-55). Karger Publishers.

86. Wood, D. L., Hoag, P. M., Donnenfeld, O. W., & Rosenfeld, L. D. (1972). Alveolar crest reduction following full and partial thickness flaps. *Journal of periodontology*, *43*(3), 141-144.

87. Yang, B. E., & Girod, S. (2014). Efficacy of Bone Healing in Calvarial Defects Using Piezoelectric Surgical Instruments. *Journal of Craniofacial Surgery*, *25*(1), 149-153.

88. Yang, H. J., Kim, R. Y., & Hwang, S. J. (2015). Pulsed Electromagnetic Fields Enhance Bone Morphogenetic Protein-2 Dependent-Bone Regeneration. Tissue Engineering Part A, 21(19-20), 2629-2637.

89. Yared, K. F. G., Zenobio, E. G., & Pacheco, W. (2006). Periodontal status of mandibular central incisors after orthodontic proclination in adults. American Journal of Orthodontics and Dentofacial Orthopedics, 130(1), 6-e1.

90. Yehya Ahmed Mostafa, M. M. (2009). Comparison of corticotomy-facilitated vs. standard tooth-movement techniques in dogs with miniscrews as anchor units. American Journal of Orthodontics and Dentofacial Orthopedics, 136 (4), 570-577.

91. Young-Seok Kim, S.-J. K.-J.-G. (2013). Effect of piezopuncture on tooth movement and bone remodeling in dogs. American Journal of Orthodontics and Dentofacial Orthopedics, 144 (1), 23-31.

92. Yu, H., Jiao, F., Wang, B., & Shen, S. G. (2013). Piezoelectric decortication applied in periodontally accelerated osteogenic orthodontics. *Journal of Craniofacial Surgery*, *24*(5), 1750-1752.

93. Zhang, J., Xu, H., Han, Z., Chen, P., Yu, Q., Lei, Y., ... & Tian, J. (2017). Pulsed electromagnetic field inhibits RANKL-dependent osteoclastic differentiation in RAW264. 7 cells through the Ca 2+-calcineurin-NFATc1 signaling pathway. Biochemical and biophysical research communications, 482(2), 289-295.

94. Zhang, J., Zhang, A. M., Zhang, Z. M., Jia, J. L., Sui, X. X., Yu, L. R., & Liu, H. T. (2017). Efficacy of combined orthodontic-periodontic treatment for patients with periodontitis and its effect on inflammatory cytokines: A comparative study. *American Journal of Orthodontics and Dentofacial Orthopedics*, *152*(4), 494-500.

**Data**

| Sample # | Group | Day Grp | Clinical Tooth Movement (mm) | CBCT Intensity Difference (I) | CBCT Tooth Distance (mm) | CBCT posterior shift (mm) | Histology Bone Density Difference % |
|---|---|---|---|---|---|---|---|
| 1 | Piezo | 7 | 1.0 | 16.8 | 0.0 | 0.1 | 10.0 |
| 2 | PEMF | 49 | 1.1 | x | x | 0.7 | -0.5 |
| 3 | Control | 7 | 0.8 | x | x | x | 18.9 |
| 4 | Control | 7 | 0.5 | 11.1 | 0.0 | 0.0 | x |
| 5 | Piezo | 21 | 1.8 | 11.5 | 0.4 | 0.3 | 9.0 |
| 6 | Control | 49 | 1.3 | 33.6 | 0.5 | 0.6 | -3.7 |
| 7 | Piezo | 49 | 1.9 | 46.3 | 2.4 | 0.7 | 12.0 |
| 8 | Piezo | 49 | 3.4 | 96.7 | 2.8 | 0.9 | 26.2 |
| 9 | Control | 21 | 1.2 | -5.8 | 0.0 | 0.3 | 17.1 |
| 10 | Control | 21 | 0.6 | 10.6 | 0.4 | 0.0 | 4.3 |
| 11 | PEMF | 49 | 2.3 | 22.6 | 0.4 | 0.7 | 2.7 |
| 12 | PEMF | 21 | 0.6 | 9.1 | 0.0 | 0.3 | 3.9 |
| 13 | PEMF | 49 | 2.1 | 66.1 | 2.7 | 0.9 | 30.7 |
| 14 | Control | 49 | x | x | x |  | x |
| 15 | Propel | 49 | 3.2 | 45.5 | 4.0 | 1.0 | 16.5 |
| 16 | Propel | 49 | 3.1 | 48.1 | 2.6 | 0.7 | 6.6 |
| 17 | Propel | 7 | 0.9 | 19.2 | 0.0 | 0.0 | 17.4 |
| 18 | Piezo | 49 | 3.7 | 44.4 | 3.4 | 0.5 | 4.2 |
| 19 | Control | 7 | 0.5 | -3.8 | 0.0 | 0.0 | 12.1 |
| 20 | Control | 21 | 1.2 | 6.5 | 0.0 | 0.3 | 3.4 |
| 21 | PEMF | 21 |  | 3.3 | 0.7 | 0.3 | 6.8 |
| 22 | PEMF | 21 | -0.2 | 10.5 | 0.0 | 0.4 | 9.1 |
| 23 | PEMF | 49 | 2.3 | 15.8 | 0.6 | 0.4 | 6.0 |
| 24 | Propel | 49 | 1.6 | 22.2 | 0.6 | 0.7 | 21.5 |
| 25 | Propel | 49 | 2.1 | 65.4 | 1.5 | 1.4 | 21.7 |
| 26 | PEMF | 49 | 2.5 | 12.5 | 1.7 | 0.7 | 13.1 |
| 27 | Propel | 21 | -0.1 | 36.7 | 0.0 | 0.3 | 41.8 |
| 28 | Propel | 49 | 2.8 | 44.0 | 2.5 | 0.7 | 1.6 |
| 29 | Control | 49 | 4.2 | 10.2 | 3.2 | 0.6 | 12.8 |
| 30 | Piezo | 49 | 2.6 | x | x | 0.6 | 38.4 |

| Sample # | Group | Day Grp | Clinical Tooth Movement (mm) | CBCT Intensity Difference (I) | CBCT Tooth Distance (mm) | CBCT posterior shift (mm) | Histology Bone Density Difference % |
|---|---|---|---|---|---|---|---|
| 31 | Piezo | 49 | x | x | x | 0.6 | 24.5 |
| 32 | Control | 49 | x | 31.0 | 3.6 | 0.5 | 9.7 |
| 33 | Piezo | 21 | 1.5 | x | x | 0.6 | 37.7 |
| 34 | Piezo | 21 | 1.0 | 69.9 | 0.8 | 0.2 | 32.2 |
| 35 | Control | 49 | 3.5 | 31.8 | 1.2 | 0.5 | 0.0 |
| 36 | PEMF | 7 | 0.1 | 4.9 | 0.1 | 0.1 | 8.7 |
| 37 | PEMF | 7 | 0.2 | 8.9 | 0.0 | 0.1 | 6.9 |
| 38 | Propel | 21 | 1.1 | 23.9 | 2.3 | 0.3 | 13.6 |
| 39 | Propel | 7 | 0.1 | x | x | 0.0 | 1.2 |
| 40 | Porpel | 7 | 0.1 | 1.5 | 0.1 | 0.0 | 0.4 |
| 41 | Propel | 7 | 0.7 | 17.6 | 0.2 | 0.1 | 29.0 |
| 42 | Propel | 7 | 0.8 | 25.3 | 0.1 | 0.1 | 26.4 |
| 43 | PEMF | 7 | 1.0 | 15.6 | 0.1 | 0.1 | 9.7 |
| 44 | PEMF | 7 | 1.5 | 21.1 | 0.3 | 0.2 | 37.9 |
| 45 | Piezo | 7 | 0.5 | 12.4 | 0.1 | 0.2 | 24.2 |
| 46 | Piezo | 7 | 0.9 | 0.4 | 0.0 | 0.0 | 20.7 |
| 47 | Piezo | 7 | 0.3 | 3.8 | 0.1 | 0.2 | 15.5 |
| 48 | Piezo | 21 | 0.9 | 49.6 | 0.6 | 0.3 | 14.3 |
| 49 | Piezo | 21 | x | x | x | 0.4 | 10.1 |
| 50 | Piezo | 7 | 0.1 | 3.8 | 0.0 | x | 13.8 |
| 51 | PEMF | 7 | 1.0 | 14.4 | 0.4 | 0.3 | 10.8 |
| 52 | PEMF | 21 | 1.4 | 34.9 | 1.7 | 0.2 | 18.4 |
| 53 | Control | 7 | 1.2 | 14.1 | 0.2 | 0.0 | 22.1 |
| 54 | Control | 7 | 0.2 | 14.9 | 0.4 | 0.2 | 28.0 |
| 55 | Propel | 21 | 1.5 | 26.1 | 2.2 | 0.4 | 19.6 |
| 56 | Propel | 21 | 0.7 | 9.4 | 0.4 | 0.4 | 2.1 |
| 57 | Propel | 21 | 1.3 | 14.1 | 0.3 | 0.3 | 4.7 |
| 58 | PEMF | 21 | 1.4 | 15.1 | 0.5 | 0.3 | 2.9 |
| 59 | Piezo | 21 | 0.5 | 25.8 | 0.0 | 0.3 | 9.0 |
| 60 | Control | 21 | 0.6 | 9.8 | 0.6 | 0.3 | 0.9 |
| 61 | Control | 21 | 2.0 | 10.3 | 1.1 | 0.3 | 8.9 |