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# Comparison of the Effects of Three Surgical Techniques on the Rate of Orthodontic Tooth Movement in a Rat Model

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# Comparison of the Effects of Three Surgical Techniques on the Rate of Orthodontic Tooth Movement in a Rat Model

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# **APPROVAL PAGE**

Master of Dental Science

Comparison of the Effects of Three Surgical Techniques on the Rate of  
Orthodontic Tooth Movement in a Rat Model

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# TABLE OF CONTENTS

<u>Title</u>	<u>Page</u>
Title Page	i
Approval Page	ii
Table of Contents	iii
Abstract	v
Chapter I – Introduction	1
Orthodontic Treatment and the Rate of Tooth Movement	1
Surgical Approaches and the Regional Acceleratory Phenomenon	3
Corticotomy	6
Corticision	13
Piezocision	15
The Effect of a Full Mucoperiosteal Flap on the Regional Acceleratory Phenomenon and Tooth movement	19
Orthodontic Tooth Movement and Root Resorption	20
Orthodontic Tooth Movement in a Rat Model	21
Rationale and Objectives	24
Chapter II – Hypothesis and Aims	26
Hypothesis	26
Specific Aims and Objectives	27
Chapter III – Materials and Methods	28
Experimental Animals	28
Experimental Design	28
Method of Orthodontic Force Application	29
Application of Corticotomy	31
Application of Corticision	31
Application of Piezocision	32



Application of Fluorescent Labeling Bone Markers	32
Orthodontic Tooth Movement Measurement and MicroCT Analysis	33
Tissue Dissection and Preparation	35
Histological Staining	36
Statistics	38
Chapter IV – Results	38
The Effect of Piezocision on Tooth Movement and the Surgical Complications	39
The Effect of Corticotomy, Corticision With and Without a Full Mucoperiosteal Flap on Tooth Movement	40
The Effect of Corticotomy, Corticision With and Without a Flap on the Alveolus	45
The Effect of the Surgical Procedures on Osteoclasts	46
The Effect of the Surgical Procedures on Odontoclasts	46
The Effect of the Surgical Procedures on Root Resorption	47
Chapter V – Discussion	48
Chapter VI – Conclusions	59
Chapter VII – Figures	61
Chapter VIII – References	79

## **Abstract**

Background: The rate of orthodontic tooth movement can be influenced by surgical procedures that traumatize the bone around the teeth to induce the regional acceleratory phenomenon. These procedures include, but are not limited to, corticotomy, corticision, and piezocision. These procedures have varying amounts of invasiveness and damage to the bone which could lead to different amounts of RAP induction affecting the influence on tooth movement. The aim of this study was to evaluate the effect of three different surgical procedures, corticotomy, corticision, and piezocision, as well as, the effect of a full mucoperiosteal flap on the rate of tooth movement and alveolar response in a rat model.

Materials and Methods: Seventy-four male (six week old) Wistar rats were divided into six groups based on the surgical procedure: Tooth Movement Only (Control), Corticotomy, Corticision, Corticision with full mucoperiosteal flap, Piezocision, and Piezocision with full mucoperiosteal flap. A constant force of 10g was applied from the maxillary left first molar to the maxillary incisors using nickel-titanium springs. Surgery was performed at the time of appliance placement, at day 0. To evaluate tooth movement, polyvinylsiloxane impressions were taken and stone models made of the maxilla on days 0, 7, 14, and 21 and Feeler gauge measurements were taken on days 0, 4, 7, 11, 14, 17, and 21. Micro-computed tomography was performed on dissected maxilla on day 21 to evaluate tooth movement, alveolar response, and root resorption in all experimental groups. Static histomorphometry was performed on day 21 samples. Tartrate resistant acid phosphatase (TRAP) staining of paraffin embedded sections was done to quantify osteoclasts and odontoclasts present. Due to surgical complications in the Piezocision groups, for example, oro-antral communication and loss of first and second molars, these groups were excluded from statistical analysis. Two additional animals were added to evaluate the piezocision surgical site with no tooth movement at day 4 and 7. Hematoxylin and

Eosin (H&E) staining, TRAP staining and TUNEL assay were performed on these specimens in order to investigate the underlying pathophysiology of the complications.

Results: In the piezocision group with no tooth movement, H&E staining revealed extensive inflammatory infiltrate at day 4, decreasing by day 7. TRAP staining indicated no osteoclastic activity at day 4 although increasing by day 7. TUNEL assay demonstrated no necrosis present near the surgical site. Stone model, feeler gauge, and MicroCT measurements showed no statistical difference between groups in the amount of OTM at day 21. Bone Volume Fraction showed no statistical difference between all groups. Static histomorphometry showed no statistical difference in osteoclast and odontoclast numbers and surface parameters between all groups. Root resorption analysis indicated more root resorption in the Corticision no flap group.

Conclusions: Corticotomy and Corticision with and without a full mucoperiosteal flap groups do not show a statistically significant difference for OTM and alveolar response compared to the OTM only group or between experimental groups. Piezocision will need to be studied further to achieve better physiological OTM.

## **Chapter I: Introduction**

### **A. Orthodontic Treatment and the Rate of Tooth Movement**

The duration of orthodontic treatment has been estimated to be between 21-27 months and 25-35 months for non-extraction and extraction treatment, respectively (1, 2). Treatment time can be influenced by the complexity of the case, skill of the practitioner, appointment attendance, replacement of brackets and bands, and the rate of tooth movement (3). As treatment time is extended, the chance for risk factors of treatment such as decalcifications or white spot lesions, dental caries (4), periodontal disease (5, 6), and root resorption (7) invariably increases. Therefore, any measure to reduce orthodontic treatment time is beneficial for both patients and practitioners.

Orthodontic tooth movement (OTM) is an orchestrated process of gradual alveolar bone remodeling which couples bone resorption and apposition in response to the application of mechanical orthodontic forces. Orthodontic forces generate mechanical strains that are transmitted to the periodontal ligament and supporting alveolar bone (8). These forces produce areas of tension and pressure within the PDL causing a sterile inflammatory process and recruitment of dental and paradental cellular populations to initiate the remodeling cycle to allow OTM. Osteoclasts and osteoblasts are among these populations and are an essential part of the alveolar remodeling process. Osteoclasts are responsible for the resorption of bone and osteoblasts are necessary for bone apposition. In a coordinated process by these cells, the tooth will move by bone resorption on the compression side and bone apposition on the tension side (8). Factors affecting this cycle of remodeling could modulate the corresponding rate of tooth movement and reduce treatment times.

Teeth move, on average, 0.8-1.2mm/month when continuous forces are applied (9). The velocity of tooth movement is regulated by bone turnover, bone density and the degree of hyalinization of the PDL in response to the forces being applied. Thus, by modifying these biological rate limiting steps, it is possible to increase OTM and thus shorten the corresponding treatment. Orthodontic research has focused on three main categories to modify these rate limiting steps: pharmacological modalities, non-invasive mechanical interventions, and adjunctive surgical procedures.

Pharmacological effects with local or systemic administration of biological factors (10, 11) such as parathyroid hormone (PTH) (12), thyroxine (13), Vitamin D3, [1,25 (OH)<sub>2</sub>D<sub>3</sub>] (14, 15), and prostaglandins (16-18) have been investigated in various experiments and have been found to increase the velocity of tooth movement. Nevertheless, the problem with pharmacological approaches is their systemic nature, which may result in numerous adverse reactions such as hyperalgesia, severe root resorption, and other drug-induced systemic and local side effects (19). Although showing a future potential in increasing OTM, the complexity and systemic nature of pharmacological reactions has led to a more concerted effort in investigating other approaches.

Non-invasive mechanical interventions are an alternative approach to drug therapy that avoids side effects. These approaches include, but are not limited to: electrical currents, laser beams and various types of vibration at different frequencies. In a recent 2013 systematic review, Long et al. concluded that electrical currents and laser beams have not been shown to increase the rate of OTM in the current literature (20). The types of vibration that have been extensively researched thus far include: whole-body vibration (21), pulsed electromagnetic field driven vibration (22), resonance vibration (23), and mechanical vibration (24).

AcceleDent's<sup>TM</sup> claim that their products that produce mechanical vibration increase the rate of tooth movement have perpetuated the subject's research (24). Conversely, it has been shown that high-frequency vibration can be anabolic to trabecular bone formation in animal and human models and thus can potentially increase bone density and inhibit tooth movement (21). With respect to vibration as a topic, however, the literature is limited and there are many areas that are still lacking sufficient information. Vibration research shows much disparity in the effects and type of stimuli utilized in a number of animal and human studies.

Pharmacological and non-invasive approaches to increasing the rate of OTM have shown extremely variable degrees of success and failure. Continuing research will need to be performed to uncover the true effects of each modality. However, another promising field is the implementation of surgical methods to help increase the rate of orthodontic tooth movement.

## **B. Surgical Approaches and the Regional Acceleratory Phenomenon**

Surgical techniques to enhance the rate of OTM have gained considerable attention in the orthodontic literature. These procedures include, but are not limited to, periodontal distraction, osteotomies, corticotomies, corticision, and piezocision (25-29). By reducing alveolar resistance to tooth movement and accelerating the turnover rate of the alveolar bone by means of the regional acceleratory phenomenon, these surgical techniques have shown substantial increase in the rate of OTM and reduction in treatment times.

These surgical procedures take advantage of the theory put forth by orthopedist Harold Frost in 1983. Frost recognized that any regional noxious stimulus of sufficient magnitude can evoke a regional acceleratory phenomenon (RAP). The size of the affected region and the intensity of its response vary directly with the magnitude of that stimulus. Once the stimulus has

been evoked, RAP produces an acceleration of the vital processes of the regional soft and hard tissues which promotes the body's healing process. Frost suggested that when the noxious stimulus was directed at osseous tissue, the result was accelerated bone turnover and reduction in regional bone density, leading to a transient state of osteopenia. RAP has been shown in orthopedic medicine with the accelerated remodeling activity occurring adjacent to the site of injury in orthopedic surgery (30, 31). It has also been shown in the maxillomandibular complex. In 1994, Yaffe et al. showed that when buccal and lingual flaps were raised in adult rats there was striking resorption of the cortical bone, demonstrating a typical RAP mechanism to potentiate tissue healing (32). In 2008, Sebaoun et al. further demonstrated the RAP effect in the alveolus with selective alveolar decortications in a rat model. Their goal was to analyze the alveolar and periodontal response to decortication surgery as a function of time and proximity to the injury. It was shown that at three weeks post injury, the alveolar trabecular bone content adjacent to the injury had decreased by two-fold and PDL surface had increased by two-fold. These events had dissipated to a steady state by eleven weeks. In addition, it was demonstrated that this RAP response was localized to the area immediately adjacent to the injury (33). These findings further support the idea that RAP phenomenon does occur in the alveolar bone and that it is localized to the area of injury.

RAP induced surgical interventions have demonstrated remarkable increases in the rate of tooth movement, but at the expense of the invasiveness of the procedure. This has led to poor patient acceptance. It would be appropriate to perform the least invasive procedure possible that causes the least morbidity while maintaining the same RAP induced response shown to increase the rate of OTM. But, as stated previously by Frost, the size of the RAP response varies with the magnitude of the stimulus (31), so a less invasive procedure might not have the same effect on

OTM if there is not adequate stimulation. It has also been shown in multiple studies that the RAP effect on alveolar bone is transient, lasting only 2 to 4 weeks (34, 35). This amount of time is not enough to have a lasting effect on the overall treatment time of patients, so more than one surgical procedure would need to be performed, increasing the morbidity and invasiveness for the patient. Due to this fact, when considering patient acceptance, it is even more important to find the least invasive procedure that will maintain increased OTM (9). Therefore, it is in order to compare the different surgical modalities as well as uncovering the importance of raising an invasive full mucoperiosteal flap on the rate of OTM.

Osteotomies have been proposed which are defined as cuts through the entire thickness of bone of both buccal and lingual cortical plates and the interposed medullary bone, creating a mobilized segment of bone that can be moved through “bony block movement.” This type of procedure has been associated with many postoperative complications such as ischemic necrosis of the bone segment, wound dehiscence, and devitalization of the teeth adjacent to the osteotomy site (36). The RAP response has not been seen in osteotomies as compared to other procedures, like corticotomies. In 2009, Wang et al. compared the different bone responses of corticotomy and osteotomy assisted tooth movement in a rat model. The authors demonstrated how osteotomy created a distal distraction osteogenesis site and how corticotomy intensified the initial bone resorptive response of normal tooth movement, creating large areas of bone resorption. Wang et al. concluded that the alveolar bone adjacent to the roots in the corticotomy group underwent resorptive, replacement, and mineralization phases of recovery (37). Thus, the use of osteotomy in clinical practice or research as a method to increase OTM is essentially unfounded.



Corticotomy, Corticision, and Piezocision have been the surgical interventions of choice for study on animal and human models and will be reviewed to shed light on the similarities and differences of the procedures. Furthermore, this will build a rationale for the purpose of comparing these three modalities.

### **C. Corticotomy**

The corticotomy procedure is a surgical technique in which a fissure is made through the buccal and/or lingual cortical plates that surround the tooth. This procedure involves elevating a full mucoperiosteal flap to have access to the bone that will be traumatized. Hassan et al. reviewed the literature on the corticotomy-assisted orthodontic treatment and provided some indications and clinical applications for the use of the procedure in practice. Uses include resolving crowding and shortening treatment time, accelerating canine retraction after premolar extraction, enhancing post orthodontic stability, facilitating eruption of impacted teeth, facilitating slow orthodontic expansion, treating molar intrusion and open bite correction, and manipulating anchorage (38).

The use of corticotomy to correct malocclusions was first introduced by Cunningham in 1893 at the Chicago Dental Congress where he presented “Luxation or Immediate Method in Treatment of Irregular Teeth.” He proposed making linear cuts in the cortical plates that surround the teeth as a means of mobilizing teeth for immediate movement (39). This concept of traumatizing the bone was again explored 67 years later in 1959 by Heinrich Kole. Kole’s original technique was to address the shortcomings of osteotomies, specifically maintaining the blood supply to the area of the procedure. The technique involved elevating a full mucoperiosteal flap both labially and lingually from the gingival margin to below the teeth

apices with a combination of interradicular corticotomies and supra-apical osteotomies. The corticotomy cuts extended through the cortical bone, just barely penetrating into the medullary bone. Kole believed that the major resistance to tooth movement comes from the cortical bone. Thus, the corticotomy served to remove this constraint. It was theorized that corticotomies allowed one to move blocks of bone with the teeth rather than moving only individual teeth (29).

Kole presented cases that took 12 weeks or less to finish without adverse side effects such as root resorption, devitalization, or pocket formation (29). However, despite showing significant decrease in treatment time, the invasive nature of the technique prevented it from becoming mainstream in the orthodontic clinic. From the time of Kole, progressing up to 1990, many authors continued to build upon and modify the corticotomy technique, publishing results demonstrating similar increases in the rate of OTM (40-43). In 1991, Suya reported on corticotomy facilitated orthodontic treatment on 395 patients. This was similar to Kole's technique, but he replaced the supraapical osteotomy with a corticotomy cut. He demonstrated cases finishing in less than 12 months and described his technique as being less painful than past techniques and having less root resorption than conventional orthodontics (44). He contributed the tooth movement to bony block movement which coincided with Kole's belief.

Wilcko and Wilcko have popularized the application of corticotomy with numerous case reports showing an impressive increased rate of tooth movement and reduction in treatment times. In 1999, Wilcko used similar techniques as described by Suya and orthodontic adjustments were made every two weeks. From bonding to debonding, an adult case finished in 18 weeks and an adolescent case finished in 12 weeks. Pre- and post-treatment computed tomography scans were performed and compared indicating significant dehiscences over the prominences of the roots of the teeth in which expansion had taken place. CT scans also

demonstrated a demineralization response to the corticotomy that was suggestive of RAP as opposed to bony block movement (45). In consideration of these findings, Wilcko adjusted the corticotomy technique and added alveolar bone augmentation before primary flap closure to increase the alveolar bone thickness and reduce the dehiscences seen in the previous cases. Their protocol was introduced as Accelerated Osteogenic Orthodontics<sup>TM</sup> (AOO<sup>TM</sup>) or Wilckodontics treatment. It later was modified to the Periodontally Accelerated Osteogenic Orthodontics (PAOO) (25, 46).

In 2001, they published two case reports. The first was a 24 year old man with Class I, severely crowded malocclusion and a constricted maxilla with posterior crossbites. The second was a 17 year old female with Class I, moderately to severely crowded malocclusion. The new surgical technique was employed and consisted of buccal and lingual full-thickness flaps, selective partial decortications of the cortical plates, concomitant bone augmentation, and primary flap closure. Total treatment time was approximately 6 months and 2 weeks. Fifteen months after surgery, flaps were raised again to examine underlying alveolar bone. It revealed good maintenance of the height of the alveolar crest and an increased thickness in the buccal bone, and no dehiscences. Again, they contributed this rapid tooth movement to the catabolic phase of RAP instead of bony block movement (47).

In recent years, the corticotomy technique has been studied in different animal models to obtain a more accurate way of quantifying the rate of tooth movement and understanding the biological mechanisms underlying the technique, rather than relying on case reports from selected patients. In 2007, Cho et al., reported on the effect of cortical activation on OTM in the maxilla and mandible in two beagle dogs. Corticotomy was performed by making 12 holes in the right cortical plate, in the fashion recommended by Wilcko. Then, the third premolars on

both treated and control sides were protracted. Cho et al. concluded that after 8 weeks, the cortical activated side showed four times as much movement in the maxilla and two times as much movement in the mandible as compared to the controls. Histologically, they showed an increase in cellular activity as compared to the control where there was increase in both resorptive cells like osteoclasts and formative cells like fibroblasts, cementoblasts, and osteoblasts (48).

Iino et al., in 2007, also reported on OTM and alveolar bone reaction after corticotomy in 12 beagle dogs. The corticotomy procedure involved flap reflection and 1 mm vertical and subapical horizontal cut lines to the alveolar bone around the third premolar. Results showed that tooth movement velocities from week one and week two were significantly faster on the corticotomy side compared to the sham control side. In fact, distances were approximately doubled at week one, two, and four. Histological evaluation showed that hyalinization of the PDL was only observed after 1 week in corticotomy groups compared to its occurrence from week one to week four in the sham groups. This coincided with the traditional lag phase of tooth movement. Also, tartrate resistant acid phosphatase positive cells (osteoclasts) in the experimental side were increased in the corticotomy group at an early time on the alveolar wall and in the bone marrow cavities. This led to the conclusion by Iino et al. that corticotomy does cause a rapid alveolar bone reaction leading to less hyalinization and increased tooth movement (49).

Teixeira et al., in 2010, further demonstrated the relationship of corticotomy and increased rate of OTM and related the process to cytokine expression. Sprague-Dawley rats were divided into 4 groups: a control, orthodontic force with spring, orthodontic force and soft tissue flap, and orthodontic force with a soft tissue flap and shallow perforations of the buccal

cortical plate. The cortical plate was perforated 3 times, 5mm mesial to the maxillary left first molar. The perforations were approximately .25mm in diameter and .25mm in depth and were performed using a round bur and hand-piece. Rats from each group were used for gene expression studies, microCT analysis, fluorescent studies, and demineralized histological studies. Results showed the perforation groups had significantly higher mean tooth movement compared to that of the orthodontic force alone and orthodontic force and flap groups. This increased rate of OTM also corresponded with a statistically significant increase in the expression of 21 cytokines/cytokine receptors, showing almost a 2-fold increase. There was also a statistically significant increase in osteoclast activity, the rate of bone remodeling, and a decrease in generalized osteoporosity when examining the microCT and fluorescent studies. The bone volume fraction (BV/TV) showed a significant decrease around all maxillary left molars. In conclusion, Teixeira et al. demonstrated that cortical perforations increase cytokine expression which most likely induces an increased inflammatory response that recruits osteoclasts and osteoblasts to increase bone turnover and induce osteopenia to increase the rate of overall tooth movement (35).

In a recent 2011 publication, Baloul et al. examined selective alveolar decortication and its ability to increase OTM and induce osteoclastogenesis and bone remodeling. They divided Sprague-Dawley rats into three groups: a selective alveolar decortication alone group, a tooth movement alone group, and a combined selective alveolar decortications and tooth movement group. Selective alveolar decortications consisted of reflection of a full thickness flap around the buccal and palatal surfaces of the maxillary left first molar. Five decortication marks were made on the buccal and palatal surfaces of cortical bone using a slow speed hand piece making 0.25mm by 0.25mm holes. The first molars were protracted with 25-gram springs. Faxitron

analysis, microCT, and quantitative real-time polymerase chain reaction (PCR) of expressed mRNAs were performed at day 0, 3, 7, 14, 21, 28, and 42 days to examine changes in all groups. Measurement of the magnitude and rate of tooth movement illustrated that the amount of tooth movement for the combined selective alveolar decortication and tooth movement group had steady increases beginning from day 0 to day 21 and demonstrated similar magnitudes of movement to the tooth movement alone group between 28 and 42 days. The rate of tooth movement in the selective alveolar decortication with tooth movement group illustrated an early, and sharp increase in the OTM compared to the tooth movement group in the first 7 days, where the tooth movement alone group showed a similar increase in tooth movement later, at the 14 day mark. Thus, the alveolar decortication procedure maintains the rate of tooth movement at a high steady level during the initial force application for part of the tooth movement. However, the effect appears to diminish at 28 days demonstrating corticotomy's transient effect on the bone and OTM. MicroCT analysis demonstrated a significant decrease in bone volume and bone mineral content in the selective alveolar decortication with tooth movement group as compared to the tooth movement alone group at day 7. PCR analysis showed corresponding increases in osteoclastic cells and key osteoclastic regulatory cytokines indicating increased osteoclastogenesis in the selective alveolar decortication with tooth movement group. Osteoblastic markers were also identified showing an increase in osteoblastic activity. This suggests an induction of bone formation by selective alveolar decortication. In conclusion, these results further demonstrate the effects of corticotomy on the RAP response of increased bone turnover (resorption and formation of bone) and reversible osteopenia that it can induce to increase the rate of OTM. However, these phenomena appear to be transient in nature (34).

Corticotomy's effect on bone has been shown to be transient, lasting only for a limited period of time. It has been shown in previous studies that the tooth movement rates approach control values after approximately 3-7 weeks (48, 49). As such, it is difficult to understand how treatment can be accelerated by 14-21 months with a single corticotomy, as shown in the numerous case reports mentioned above. If the teeth need to be moved more orthodontically and the rate of movement has slowed due to the decreased bone turnover then corticotomy procedures would need to be performed again to start RAP again to maintain the increased tooth movement.

In 2010, Sanjideh et al. examined this concept when demonstrating the effects of a second corticotomy on a foxhound model to determine if a second corticotomy procedure produced more tooth movement than a single procedure. After 4 weeks from the initial corticotomy and tooth movement, a second corticotomy was performed. Overall, this study concluded that alveolar corticotomy does increase the rate of tooth movement by nearly 100%, which is consistent with other studies (48, 49). Another conclusion was drawn that a second corticotomy maintained higher rates of orthodontic tooth movement over a long duration and produced greater overall tooth movement than performing just one corticotomy at the beginning of the treatment. However, the authors stressed that the difference between the two groups was small and that it does not justify a second procedure (9, 50).

The literature clearly demonstrates that corticotomies induce a RAP response in the affected bone of increased bone turnover and reversible osteopenia that increases the rate of OTM. In summary, it has been demonstrated that corticotomies increase the rate of tooth movement approximately two-fold. On the other hand, the procedure does have disadvantages. It has been estimated, based on research on dogs, that the effects of corticotomies are limited to

only 2-3 months in humans during which 4-6mm of tooth movement might be expected (9). The transient effects of corticotomies might not produce enough of a RAP response to significantly decrease the overall average treatment time of 18-30 months depending on the type of treatment (1, 2). If it does not have a clinically significant effect on the overall treatment time during the first procedure, a second one might be in order. There have also been several reports of adverse effects to the periodontium. While some reports indicate no problems, others have shown mild interdental bone loss, loss of attached gingivae and periodontal defects (38). Considering the invasiveness of corticotomies, chances for periodontal defects and the possibility of multiple procedures to complete treatment, patients will be wary of undergoing this type of therapy. Patients who desire or need a shortened treatment period will need to decide whether the benefit is worth the potential drawbacks. This provides reason for the investigation of new techniques that are less invasive, do not require flap surgeries, and are kinder to patients (and their periodontium) (9).

#### **D. Corticision**

Corticision is a flapless procedure that can be performed under local anesthesia with minor or no morbidity. The procedure involves a reinforced scalpel used as a thin chisel to separate the interproximal cortices transmucosally (26). The blade is then tapped with a mallet to penetrate the cancellous bone with the goal of inducing enough injury in the cortex and cancellous bone to promote RAP and increase the rate of tooth movement when orthodontic force is applied (51). The literature on corticision in animal or human studies is far less extensive than that of corticotomy literature, with most pertaining to case reports.



In 2009, Kim et al. investigated the biologic effects of corticision on alveolar remodeling in orthodontic tooth movement. A feline tooth movement model was divided into three groups: orthodontic force only (control), orthodontic force plus corticision, and orthodontic force plus corticision and periodic mobilization. At day 7 there was less hyalinized tissue and more viable PDL cells in the corticision group compared to the control, similar to the findings of Iino et al. By day 14, there were large resorption cavities with the recruitment of osteoclast like cells, and by day 28 the mean apposition area in histomorphometric analysis showed a 3.5 times greater mean apposition rate. In conclusion, Kim et al. showed corticision's ability to stimulate orthodontic tooth movement in 28 days by accelerating alveolar bone remodeling (26).

The same group, Kim et al., reported on their investigation into the combined effects of corticision and lower level laser therapy (LLLT) on the tooth movement rate and paradental remodeling in a beagle model. The researchers acknowledged that corticision can only produce a RAP response for a transient period, as shown in Kim's previous study. Thus, in conjunction with another minimally invasive procedure (LLLT), they aimed to improve the RAP intensity and duration. Twelve beagles were divided into four groups: orthodontic force only, orthodontic force plus corticision, orthodontic force plus LLLT, and orthodontic force plus corticision and LLLT. Corticision was performed on the mesio-buccal, distobuccal, mesio-palatal, and disto-palatal side of the maxillary second premolars and then protracted. At week 8, compared to the control group, mesial movement of the second premolars in the corticision group increased 3.75-fold. There was actually a decreased rate in OTM with combined corticision and LLLT, for reasons that could not be identified (52). The importance of this study in regards to the proposed research project is that corticision alone does induce a RAP response and increase OTM.

A recent unpublished study at the University of Connecticut, Orthodontic Division by Vaziri et al. observed that rats subjected to corticision at the beginning of the experiment and one week after the application of force demonstrated accelerated molar mesialization compared to control animals (53). However, in a subsequent published study at the University of Connecticut Orthodontic Division, Murphy et al. compared corticision groups with different magnitudes of forces and it was found that there was no significant difference in tooth movement compared to control groups (54).

The advantage of the corticision procedure being less invasive and the literature illustrating instances of increases in alveolar remodeling and OTM presents a promising field of study and potential use in clinical practice. Thus, further investigation is needed to compare surgical modalities and determine the importance of raising a full mucoperiosteal flap when compared to the corticotomy procedure. This will serve to provide patients with the best treatment to decrease orthodontic treatment duration.

#### **E. Piezocision**

Piezoelectric surgery utilizes variable-frequency ultrasonic micro-vibrations as a powerful and efficacious surgical force that is able to cut bone without uncontrollable trauma (27, 55). Micro-movements of 60-200 $\mu$ m ensure the selective cutting of mineralized hard tissue. The frequency of the oscillations lies between 22-29kHz, which makes it possible to avoid soft tissue, preventing unwanted damage to blood vessels, nerves, and membranes that are cut at frequencies higher than 50kHz (56). This technique is utilized in numerous medical fields. In dentistry, it has become commonplace to use piezoelectric surgery in periodontal surgeries such as sinus augmentations to avoid perforating the sinus membrane. It has also been used in ridge

expansion techniques for placement of implants instead of osteotomies that tend to lead to bone fractures and defects of the implant site. The fundamental idea on which piezoelectric surgery is based is the use of a surgical force that is able to cut bone according to the requirements of the case, with powerful and precise energy and without excessive trauma. The piezosurgical insert allows for cuts that are sub-millimeter in width and the physiological cooling liquid contributes to a bloodless surgical field (57).

Research of piezocision in increasing the rate of orthodontic tooth movement is limited but it theoretically can offer many advantages to the patient compared to corticotomy and corticision. Surgical control for piezosurgery has been described as effortless because the force necessary to obtain a cut by the operator is much less than what is needed for corticotomies using a bur or corticisions using a reinforced scalpel and mallet. It allows for precise application of the cuts at the correct location and depth, controlling the amount of penetration through the cortical bone and into cancellous bone (27).

Patient discomfort appears to be reduced, resulting in higher acceptance rates for the procedure. Also, patients appreciate the elimination of a high speed hand-piece (55). Increasing patient comfort and ease of use for the clinician would be an excellent practice builder and increase the acceptance into clinical use in everyday orthodontics. More extensive research needs to be performed to determine if piezocision will in fact induce enough of a RAP response to increase OTM.

Verocelotti and Podesta evaluated eight patients with multiple types and severities of malocclusions. With these patients, they used a technique called monocortical tooth dislocation and ligament distraction (MTDLD). The goal was to increase the rate of tooth movement and prevent damage to periodontal tissues in adult patients who need both of these. The technique

involves vertical and horizontal microsurgical corticotomies performed around each tooth with a piezosurgical instrument to eliminate cortical bone resistance. This is immediately followed by application of strong orthodontic forces producing rapid dislocation of the root and cortical bone together. The greatest amount of dental movement occurs in the first third of the overall treatment time. From their case series, piezocision was performed with conventional flap elevation on the buccal or palatal cortical plate corresponding with the direction of desired tooth movement. The authors concluded that compared to conventional orthodontic therapy, the average treatment time was reduced in the mandible and maxilla by 60% and 70% respectively, and no serious periodontal complications were encountered during and one year after treatment (58).

Dibart et al. more recently reported in two cases on the rapid treatment with piezocision in patients with Class II malocclusions. It was mentioned that flap raising as performed in Wilcko's PAOO and Verocelotti's MTDLD was quite invasive, with the potential to create post operative discomfort and complications. For these reasons, flap raising is not mainstream in the dental community. They also summarized the drawbacks of corticision presented by Kim et al. (52). The first drawback is that going directly through the attached gingiva does not allow for supplemental periodontal grafting to correct inadequacies. Furthermore, the malleting that is involved in corticision to allow the scalpel to penetrate the cortical bone has been shown to cause dizziness postoperatively. Dibart et al., thus, produced a novel technique utilizing piezocision combined with selective tunneling that allows for hard or soft tissue grafting and piezoelectric incisions. They claim that grafting makes the procedure versatile and incisions are only placed on the buccal surface, eliminating the need to access the lingual or palatal alveolar bone (27).

The first patient was a 31-year old woman with maxillary and mandibular crowding who had piezocision surgery performed 1 week after orthodontic appliance placement. Ten vertical interproximal incisions were made through the periosteum below the interdental papillae on the buccal aspect of the maxilla and mandible. Incisions were made between every tooth, except on the mandibular anterior teeth in which every other interproximal was involved to increase retention of the bone graft as grafts were needed there most often. A piezosurgery knife was used to create cortical alveolar incisions to a depth of 3mm and demineralized freeze-dried bone allograft was placed in appropriate areas. The patient had uneventful healing, no swelling, bruising or major discomfort and active treatment time was 8 months. The second patient had similar dental characteristics and similar treatment procedures were used and active treatment time lasted 8 months and 2 weeks and ideal orthodontic outcomes were achieved (27).

In an ongoing randomized clinical trial at the University of Connecticut Orthodontics Clinics, piezocision is being investigated for its ability to affect the alignment of mandibular incisors and if the treatment is quicker than conventional braces alone. A technique very similar to Dibart et al. was utilized on the mandibular incisors, with the only exception being there was no bone grafting performed. Preliminary results indicate that the piezocision group has not shown an increase in the rate of OTM for the alignment of the mandibular incisors (59).

Dibart et al. recently attempted to show the effects of piezocision in a Sprague-Dawley rat model. They divided their groups into no treatment, tooth movement alone, piezocision alone, and tooth movement with piezocision. Piezocision was performed by making vertical incisions into the mesio-palatal and disto-palatal gingiva adjacent to the left maxillary first molar with a scalpel blade. This was followed by insertion of the piezotome blade to create an alveolar bone injury of 0.5mm in depth. Results showed that in the piezocision only and piezocision and

tooth movement groups the bone content significantly decreased and osteoclastic activity significantly increased compared to the other groups. Also, there was significant increase of two-fold in the piezocision and tooth movement group compared to the tooth movement only group. This further suggests that piezocision does stimulate an increase in bone turnover activity and OTM (60).

The Dibart et al. technique illustrates the potential piezocision has in accelerating tooth movement and correcting thin biotypes and gingival recession. However, the evidence and supporting literature is limited. More animal and human randomized clinical control trials need to be performed to justify the use of this technique in clinical practice. Cellular mechanisms need to be understood and the procedure needs to be compared to other surgically assisted tooth movement procedures to determine which provide the best mechanism for tooth movement and if they produce enough of a RAP response to provide clinically significant reductions in treatment time. Hence the rationale for comparing piezocision with other surgically assisted tooth movement procedures, i.e, corticotomy and corticision, to compare their effects at the cellular level and on tooth movement.

#### **F. The Effect of a Full Mucoperiosteal Flap on the Regional Acceleratory Phenomenon and Tooth Movement**

A full mucoperiosteal flap consists of elevating the mucosa off the underlying bone which causes the separation of the periosteum. Swapp et al. discussed the consequences of separating the periosteum and the possible sequelae of the underlying bone. They stated that the periosteum consists of most of the arterial supply and venous return of the bones in the body, specifically long bones. The flap would thus induce ischemic events and cause the medullary bone to respond to the injuries providing cells and calcium and other mineral for repair. This

would subsequently cause a RAP response with increase bone turnover and decreased bone density (61).

Yaffe et al. demonstrated this effect with mucoperiosteal flaps performed alone on the alveolus. It caused significant amounts of resorption and bone remodeling in the alveolus and a buccal and lingual flap was more effective at this than solely a buccal flap (32). Thus, elevating a full flap in conjunction with a surgical procedure would increase the trauma to the bone and increase the RAP response and possibly increase the rate of orthodontic tooth movement.

Ruso et al. performed piezocision without a flap on the buccal alveolus in foxhound dogs to evaluate tooth movement and bone response. Teeth were moved buccally and demonstrated that the piezocision group had significantly more tooth movement and less dense and mature bone than the control (62). In contrast, Swapp et al. demonstrated that a flapless decortication procedure, using a bone awl, did not affect the rate of tooth movement in foxhound dogs when the third premolars were mesialized. It was suggested that to increase the RAP process more damage may need to be created and elevating a flap would induce the response needed (61). These conflicting tooth movement studies performed with different surgical techniques provide the rationale to test the effects of surgical procedures with and without full mucoperiosteal flaps on the rate of tooth movement and alveolar response.

## **G. Orthodontic Tooth Movement and Root Resorption**

Orthodontic induced root resorption is a common, unwanted sequela of treatment. Often unavoidable, the resorptive process can be of varying degrees with a strong link to patients that have a genetic predisposition. It has been shown that length of treatment has a positive correlation with the amount of root resorption. This means that the longer the patient's treatment,

the more likely root resorption will be seen on their final radiographic images. Depending on the severity, root resorption could lead to clinical and legal implications for the patient and orthodontist. Thus, decreasing the length of treatment, specifically with surgical procedures, would most likely decrease the amount of root resorption (7).

It has been theorized that bone density has an important role in the process of root resorption and that bone and roots have similar densities and will undergo the resorptive processes at a similar rate. So it can be assumed that if the density of the bone in which the root was moving was of lower density, there could possibly be less root resorption. This has been shown in rat studies in which calcium deficient rats with decreased alveolar densities have lower levels of root resorption (63, 64). Conversely, when teeth are moved in close proximity to dense alveolar cortical bone the roots undergo more resorption than teeth moved through less dense trabecular bone (65). The regional acceleratory phenomenon has been shown to produce a transient state of osteopenia, which could be conducive to reducing resorption as well as increasing OTM (31). This provides a rationale for studying the effects of these surgical procedures and possibly implementing them in clinical practice.

#### **H. Orthodontic Tooth Movement in a Rat Model**

Several animal models have been designed to study tissue responses to mechanical loading during orthodontic tooth movement, which include primates, dogs, cats, rats and mice. The limitations of these animal models are due to their dissimilarity and applicative value to humans. The rat model proposed by Waldo in 1954 (66) has become the investigative workhorse for unraveling the processes of mechanotransduction and alveolar bone remodeling in orthodontic tooth movement (67). Compared with most other animals, the use of the rat has several advantages. They are relatively inexpensive, allowing for large sample numbers, they can



be housed for long periods of time, and histological preparation of the rat is easier than other models. In addition, there is greater availability of antibodies required for cellular and molecular biological techniques. Finally, rats are larger than mice, which make it easier to place orthodontic appliances. However the rat model does have its own limitations when compared to humans. The rats have denser alveolar bone, they lack osteons and have less abundant osteoid tissue. They also show structural dissimilarities in PDL fiber arrangement and tissue development during root formation. Lastly, tissue changes as a consequence of orthodontic treatment appear to be faster in rats than in humans, although their principal mechanisms are the same (67).

In a systematic review of the 153 (57% of the total tooth movement models) studies done on rats over the past twenty years, Ren et al. found that the majority of the experimental models used poorly designed force systems that lacked control over force levels and constancy over the duration of tooth movement (67).

Only four methods met Ren's inclusion criteria for a good model (67). Ren's inclusion criteria were: a force magnitude of less than 20cN; mesial movement of molars; an experimental duration greater than 2 weeks; and no extra experimental conditions, such as drug intervention. Most of the studies failed to take into account the physiology of the rat (i.e. natural distal drift of the molars and the continual eruption of the incisors), or the orthodontic appliance design was faulty. Also, the distal drift of the molars underestimates the amount of mesial movement of the molars and continual eruption of the incisors can lead to minimized control of force direction. The appliance design can be considered poor when it does not take into account the 50-fold decreased rat molar root surface area compared to humans, or it lacks a constant and continual force (67).

In the 1990's, King (68), Keeling (69), and Nixon (70) produced the only 3 articles that met all of Ren's criteria for an ideal rat model (67). Forces of 20, 40, and 60cN were used in all 3 articles. These studies were criticized for having an initial constant force, but not reactivating it, and for having forces of 40 and 60cN that could be considered too high. The appliance consisted of a 9 mm length of closed coil spring (0.006 inch Hi T; arbor diameter: 0.022 inch, Unitek, Monrovia, Calif.) suspended between a cleat bonded to the occlusal surface of the maxillary first molars and the lateral surface of the maxillary incisors. Initial force values were measured by suspending known weights from the anterior end of these coils before fixation to the incisors. Tooth movement was determined from enlarged cephalograms, and was measured from the position of a reproducible landmark on the molar cleat with respect to either zygomatic amalgam implants, or a barbed broach placed submucosally on the palate. Palatally placed barbed broaches represented a more reliable, less traumatic, and more easily executed superpositional landmark than zygomatic amalgams. There was only a 79% appliance success rate, the animals lost weight, and they extracted mandibular first and second molars. All of these factors contributed to what was considered poor overall animal care (67-70).

In response to these weaknesses Ren developed a new rat model. A split mouth design was chosen in order to account for physiologic distal drift of molars, the physiologic growth of the snout and forward movement of the incisors, continuous eruption of the incisors and potential distal tipping of the incisors used as anchorage. The experimental side was chosen randomly and the contralateral side served as the control. Stainless steel ligature wires (0.2 mm in diameter) were bent to connect all three maxillary molars as a unit and a wire Sentalloy® closed coil spring (10 cN, 0.22 mm wire diameter, 0.56 mm eyelet diameter) was attached to deliver a reproducible force of  $10 \pm 2$  cN over a range of 3-15 mm activation. The animals were placed under general

anesthesia and a transverse hole drilled through the alveolar bone and both maxillary incisors at the mid-root level to accommodate a stainless steel ligature. Upon placement, bonding and activation of the preformed appliance it was attached to the ligature wire through the snout and incisors. Intraoral measurements and radiographs were taken at 0, 1, 2, 3, 4, 8, and 12 weeks. Intraoral measurements were made from the most mesial point of the maxillary molar unit and CEJ of the ipsilateral maxillary incisor at the gingival level (67).

For the proposed study, Ren's rat model and inclusion criteria will be taken into consideration when designing a model to evaluate different surgical techniques to increase the rate of orthodontic tooth movement.

## **I. Rationale and Objectives**

Increasing the rate of orthodontic tooth movement is beneficial for the patient because it will decrease treatment time and reduce adverse effects of treatment including caries, periodontal problems and root resorption (4, 5, 7, 71). To increase the rate of tooth movement, the basic underlying biological processes of tooth movement need to be modified. The process of resorption by osteoclasts and deposition of bone by osteoblasts determine the rate of bone turnover, which in turn determines the rate of tooth movement (8). Numerous applications have been utilized to increase bone turnover, each with varying levels of success. Surgical interventions, particularly corticotomy, corticision, and piezocision, traumatize bone and induce a localized regional acceleratory phenomenon which increases the rate of bone turnover and causes a state of osteopenia. The amount of RAP response is dependent on the extent of injury to the alveolar bone (30, 31). Corticotomy, which involves a full mucoperiosteal flap, produces the most injury to the bone compared to the other two, and has been popularized through numerous successful case reports and animal studies (9, 25, 29, 33, 35, 45-49). However, the

procedure's invasiveness and adverse effects have limited its clinical use. New applications, like corticision and piezocision, have been introduced and do not require a full mucoperiosteal flap. They are less invasive and traumatizing for the patient and have been shown to induce a RAP response and increased rate of tooth movement (26, 27, 52, 55, 57, 58). It is not known if these biological or clinical effects are similar to corticotomy. Lastly, root resorption is an unwanted side effect of OTM. It has been theorized that decreasing the bone density, as does RAP induced by surgical procedures, is correlated to a decrease in root resorption (63, 64). Thus, the purpose of this study is to compare the effect of corticotomy, corticision, and piezocision and to determine the importance of a full mucoperiosteal flap on rate of orthodontic tooth movement. Another aim is to quantify alveolar bone and root changes and localize the osteoclasts responsible for the biological response and resulting tooth movement.

## **Chapter II: Hypothesis and Aims:**

### **A. Hypotheses and general objectives**

**Hypothesis 1:** We hypothesize that the rate of orthodontic tooth movement will be increased in the experimental group with corticotomy as compared to the corticision, piezocision, and control groups.

**Hypothesis 2:** We hypothesize that the rate of orthodontic tooth movement will be increased in the experimental groups with full mucoperiosteal flaps as compared to the corticision, piezocision, and control groups alone.

**Hypothesis 3:** We hypothesize that there will be increased osteoclasts, decreased bone volume fraction, decreased tissue density, and resorptive surfaces in the experimental group with corticotomy and groups with full mucoperiosteal flaps as compared to the corticision, piezocision, and control groups.

**Hypothesis 4:** We hypothesize that there will be less root resorption in the corticotomy group as compared to all experimental and control groups.

**Null Hypothesis 1:** There will be no difference in the amount of tooth movement among experimental groups and the control group.

**Null Hypothesis 2:** There will be no difference in the amount of tooth movement among groups with full mucoperiosteal flaps and groups without flaps.

**Null Hypothesis 3:** There will be no difference in osteoclast quantity, bone volume fraction, tissue density, and resorptive surface area among experimental groups and the control group.

**Null Hypothesis 4:** There will be no difference in root resorption among experimental groups and the control group.

## **B. Specific Aims/ Objectives**

**Specific Aim 1:** To determine the effect of corticotomy, corticision, and piezocision with and without a full mucoperiosteal flap on the rate of OTM at a constant force.

**Specific Aim 2:** To examine the alveolar structure after OTM at a constant force with corticotomy, corticision, and piezocision with and without a full mucoperiosteal flap.

**Specific Aim 3:** To quantify and determine the localization of osteoclasts and bone parameters during OTM at a constant force with corticotomy, corticision, and piezocision with and without a full mucoperiosteal flap.

**Specific Aim 4:** To examine the effects on root resorption after OTM at a constant force with corticotomy, corticision, and piezocision with and without a full mucoperiosteal flap.

### **Chapter III: Materials and Methods:**

#### **A. Experimental Animals**

6 week old, male Wistar rats (Charles River Laboratories International, Inc., PA, USA, body weight 150-250g) were used for the experiments. The animals were housed under normal laboratory conditions and fed transgenic crushed food diet (Bio-Serve, Frenchtown, NJ) and water ad libitum. The food was checked and changed every day. A standard 12 hour light and dark cycle was maintained. The animals were acclimatized for at least 1 week before the experiment started. The rats were weighed twice a week in order to ensure their weight. No animals lost more than 20% of their original weight and needed to be excluded from the study.

Upon completion of the experiment, all rats were euthanized by CO<sub>2</sub> inhalation, followed by cervical dislocation. All animal experimental procedures were in compliance with the guidelines in the care and use of animals in the American Journal of Physiology and the University of Connecticut Health Center.

#### **B. Experimental Design**

All experiments were performed under an institutional approved protocol for the use of animals in research (ACC#100734-0816).

In this study 89 rats were randomly divided into 6 experimental groups (14 rats in each group) with an additional Control group of 5 rats without any treatment. This study was a pilot study so a power analysis was not performed to calculate sample size. In each group, the orthodontic tooth movement and the surgical procedures were applied to the left side of the maxilla. Total duration of tooth movement was 21 days. As the study progressed there were

complications with the piezocision groups and they were not completed (see Results for description of complications). This caused the groups to be defined as the following:

Group 1: Orthodontic force only (n=14)

Group 2: Corticotomy and orthodontic force (n=14)

Group 3: Corticision and orthodontic force (n=14)

Group 4: Piezocision and orthodontic force (n=13)

Group 5: Corticision with flap raised and orthodontic force (n=14)

Group 6: Piezocision with flap raised and orthodontic force (n=3)

Group 7: Control with no orthodontic force and no tooth movement (n=5)

Two additional animals were used to evaluate the tissue reaction occurring as a consequence of the corticotomy and piezocision procedures in order to preliminarily investigate the surgery complications. Both animals had only surgery performed with no orthodontic force applied. One rat had piezocision performed on the left maxilla and corticotomy performed on the right maxilla and was sacrificed at day 4. The other animal had piezocision performed on the left side and was sacrificed on day 7.

This brought the total number of animals evaluated in the study to 81.

### **C. Method of orthodontic force application**

Animals were placed under general anesthesia with xylazine (8mg/kg) and ketamine (80mg/kg). Anesthesia was verified by the lack of response by the rat to a toe-pinch. A low force/deflection rate nickel titanium coil spring (Ultimate Wireforms, Inc., CT, USA) delivering approximately 10 grams was used for the application of orthodontic force for tooth movement. The force/deflection rate ( $F/\Delta$ ) for the spring was determined in order to calibrate the amount of force produced by activation of the spring. For the 10 gram springs, 2-4 grams was required to



activate the springs, thus the springs extend with a spring constant of 14-15 grams per mm (Figure 1) (54).

Prior to appliance delivery a 0.008 stainless steel (SS) wire was threaded through the contact between the first and second left maxillary molars. The 10 gram spring was then attached to the 0.008mm SS ligature around the first molar and ligated at the mesial surface of the maxillary first molar. Self-etching primer (Transbond Plus self etching primer, 3M Unitek) was applied to the mesio-palatal surface of the first molar, and the ligature bonded with light-cured dental adhesive resin cement (Transbond 3M Unitek) with a commercial unit (LEDemetron 1, Dentsply). A second 0.008mm SS ligature was placed around the incisors, the spring was attached and activated, and reinforced with the same bonding procedure as the molar. In addition, grooves 0.5mm from the gingiva were prepared on the distal surface of the maxillary central incisors to prevent the ligatures from dislodging from the incisor due to their lingual curvature and eruption pattern. After the ligatures were tied and cut, composite resin (Transbond XT Light Cure Adhesive Paste, 3M Unitek, Monrovia, CA) was placed over the wire to prevent slipping and gingival irritation, as well as pulpal irritation due to exposed dentin. The mandibular incisors were reduced to prevent appliance breakage (Figure 2). After appliance insertion the rats were allowed to recover in the presence of an incandescent light for warmth and the animals were returned to their cages once full ambulation and self-cleansing returned. The appliance was checked twice weekly and additional bonding material was added if necessary. On day 7 and 14 following initial placement of the appliance, the animals were anesthetized and the springs reactivated and placed more gingivally on the incisors due to their continuous eruption pattern. Only the left side of the maxilla was treated and evaluated for histological and micro-CT purposes.

#### **D. Application of Corticotomy**

For all surgical procedures, anesthesia was induced, and a 0.036in SS fabricated mouth-prop, placed between the maxillary and mandibular incisors, was used to hold the rat's mouth open. Procedures were applied at the time of orthodontic appliance placement.

The corticotomy procedure was performed similar to the technique presented by Teixeira et al. (35). A full thickness flap was elevated on palate adjacent to the left first molar, extending mesially to allow for cortical perforations. Using a slow-speed hand piece and a No. ¼ round bur, 3 shallow perforations, each 0.25mm wide and 0.25mm deep corresponding to the size of the bur, were made 5mm mesial to the left first molar under water irrigation. Drilling was performed where the 0.25mm bur was fully immersed in to the bone for every perforation (Figure 3). The flaps were then sealed with cyanoacrylate tissue adhesive (Vetbond, 3M Unitek, MN, USA). Primary closure was achieved for primary tissue healing.

#### **E. Application of Corticision**

Corticision was performed on the mesial-palatal and disto-palatal aspect of the maxillary left first molars in the corticision groups. The tip of the reinforced surgical blade (No. 11, Bard-Parker, NJ, USA) capable of making a surgical incision with a minimum thickness of 400µm was employed. The blade was positioned on the mesio-palatal and disto-palatal gingiva 0.5mm from the corresponding tooth surface at an inclination of 45-60° to the long axis of the maxillary first molar. The blade was inserted gradually penetrating the overlying gingiva, cortical bone, and cancellous bone (Figure 4) to approximately 1mm. To ensure the blade went consistently to a depth of 1mm, a composite stop was placed 1mm from the tip of the blade to stop it from penetrating past 1mm.

In the experimental group with corticision with a flap raised, the flap was raised similar to the corticotomy procedure. Then, the corticision was performed as mentioned above, excluding transmucosal entry.

#### **F. Application of Piezocision**

Piezocision was also performed on the mesio-palatal and disto-palatal aspects of the maxillary left first molars in the piezocision groups similar to Dibart et al. (60). An incision was made in the mesio-palatal and disto-palatal gingiva 0.5mm from the corresponding tooth surface with a reinforced surgical blade (No. 11, Bard-Parker, NJ, USA). The incision was 2mm long penetrating the gingiva down to the cortical bone. After the incision the gingiva was slightly elevated laterally to visualize the bone. A piezosurgery knife with a 2mm insert (OT7) (Piezosurgery Mectron, Piezosurgery Incorporated, OH, USA)), was used to create the cortical alveolar incisions to a depth of 1mm into the cortical bone, just penetrating into the cancellous bone and 2mm long (Figure 5).

In the experimental group with piezocision with a flap raised, the flap was raised similar to the corticotomy procedure. Then, the piezocision was performed as mentioned above, excluding transmucosal entry.

#### **G. Application of fluorescent labeling bone markers**

During the experiment, rats received a series of intraperitoneal injections with bone labeling markers oxytetracyclin (30 mg/kg), calcein (15 mg/kg) and alizarin red (30 mg/kg). On day 0, rats were injected with oxytetracyclin, on day 7 rats with calcein and day 14 with alizarin red. The labeling was analyzed on frozen undecalcified sections of maxillary alveolar bone.

## **H. Orthodontic Tooth Movement Measurement and Micro-CT Analysis**

Tooth movement was measured using feeler gauges on days 4, 7, 11, 14, 17, and 21. Measurements were carried out by a single examiner and were taken by placing the feeler gauges perpendicular to the occlusal surface interproximally between the maxillary first and second molar. The amount of tooth movement was the gauge that fit in the space without displacing the teeth apart more than present.

Tooth movement was also measured by taking Polyvinylsiloxane (PVS) impression of the maxillary left first, second and third molars on day 0, 7, 14, and 21 (Figure 6). The impressions were used to fabricate dental die stone models (Figure 6). The models were placed on a table in putty in order to have the occlusal plane parallel to the table with a millimeter ruler beside it. A digital photograph was taken parallel to the occlusal plane of the model and the ruler. This image was then uploaded into ImageJ (Image Processing and Analysis in Java, NIH). Distance measurements were then taken from the distal groove of the first molar to the mesial groove of the second molar with the millimeter ruler as reference for the distance (Figure 6). Measurements were taken and subtracted from one another in order to illustrate weekly, biweekly, and final tooth movement measurements in each group. These time periods were from day 0-7, 0-14, 0-21, 7-14, 7-21, 14-21. Four models were measured a second time greater than two weeks from the first measurement in each group for each day to calculate Intraclass Correlation Coefficient to illustrate accuracy of measurements.

MicroCT analysis was performed on the sacrificed and dissected rat maxillae at the microCT facility at the University of Connecticut Health Center on each animal on day 21 (Figure 7).

Scanning was performed at 55 kV and 145 mA, collecting 1,000 projections per rotation at 300 millisecond integration times. Three-dimensional images were constructed using standard convolution and back projection algorithms with Shepp and Logan filtering and rendered within 12.3 mm field of view at discrete density of 578,704 voxel/mm<sup>3</sup> (isometric 12mm voxels).

The serial images were used for quantitative analysis of alveolar bone changes occurring in the region of the maxillary 1<sup>st</sup> molar. The region of interest (ROI) for the alveolar bone analysis was defined vertically as the most occlusal point of the furcation to the apex of the maxillary roots. Transversely, it roughly included the square (Figure 20) with one side connecting the points of the most mesial part of the distobuccal root with the most mesial part of the disto-palatal root and the other sides extending to the points of the most distal parts of the mesio-buccal and mesio-palatal roots.

Parameters that were studied include bone volume (BV), tissue volume (TV), bone volume fraction (BVF), apparent density (AD) and tissue density (TD). Bone volume represents the volume of voxels above a specific threshold considered to represent mineralized tissue and therefore representative of bone. Tissue volume is the total volume of tissue enclosed by the region of interest. Bone volume fraction is determined by the ratio of bone volume to total volume. This parameter indicates the percentage of the total volume that is made up of bone. Tissue density defines what is considered to be bone. Apparent density represents the mean value of all voxels within the region of interest including bone and background.

Tooth movement was evaluated by connecting the most distal point of the first molar (M1) and the most mesial point of the second molar (M2) and the intermolar distance (M1-M2 distance) was used to evaluate the amount of tooth movement.

For microCT analysis of root resorption, only the distobuccal roots of the first maxillary left molars were segmented and customized software measured their volumes by contouring the root surface on 2-D slices. The crowns were not included in the measurements and the separation line (crown–root) was placed at the points demarcating the cementoenamel junction (CEJ).

Tissue volume (TV) included all the structures within the root including the pulp. Root volume (RV) was restricted to the root dentin and RV/TV ratio was used as a representation of root resorption for the statistical tests.

### **I. Tissue Dissection and preparation**

Following euthanasia at day 21, after decapitation, the mandibles were removed. The maxilla was then hemisected, and cleansed of soft tissues and muscles. The hemisected maxilla subsequently was placed in 10% formalin for five days at 4°C with constant agitation. Following fixation, springs were removed and the samples were then sent for MicroCT imaging and analysis. After the analysis, half of the specimens (7 in each group) were briefly washed with tap water and decalcified in 14% EDTA for 3 weeks and then processed for standard paraffin embedding. Serial sagittal sections of the maxillae were performed and stained with hematoxylin and eosin (H&E) and TRAP. The other half of the specimens were processed for frozen embedding. The samples were placed in 30% sucrose overnight and the following day, the crowns were trimmed at the cementoenamel junctions and the maxillae were embedded in Thermo Shandon medium, flash frozen in a solution of 2-methylbutane and stored at -20°C. The 5µm thick frozen sagittal undecalcified sections were obtained using a Leica CM1900 Cryostat (D-69226; Leica, Inc., Nussloch, Germany). In order to analyze the sections by fluorescent microscopy, they were rehydrated in PBS and mounted with glycerol/PBS suspension.

Fluorescent imaging was obtained by using the appropriate filter cubes (FITC, Texas Red and APC) on the Zeiss Axiovert 200 Microscope equipped with motorized stage and digital camera.

Three sections of the first maxillary molars per each experimental animal were scanned at 10x magnification and then stained with Von Kossa to assess the alveolar bone mineralization in each section.

For quantitative analysis of bone labeling (bone mineralization/apposition rate), the alveolar bone in furcation area was divided in 4 equal quadrants in the sagittal plane and only the coronal quadrant adjacent to the mesial root was considered to be a bone forming area or region of interest (ROI) (Figure 8).

#### **J. Histological Staining**

Prior to histological staining, tissue sections were deparaffinized with xylene and rehydrated with decreasing concentrations of ethanol and then washed in deionized water. The sections were then stained with hematoxylin for 3 minutes and eosin for a couple of seconds and dehydrated in 95% and 100% ethanol.

Staining for tartrate-resistant acid phosphatase (TRAP) activity was performed using an acid phosphatase leukocyte kit (386-A1, Sigma Chemical, St Louis MO) according to the manufacturer's instructions. Osteoclasts were considered as TRAP positive multinucleated cells (2+ nuclei) and were counted on the alveolar bone surface of the compression side of the disto-buccal root. Histomorphometry analyses were carried out using Osteomeasure Software (Osteometrics Inc, Decatur GA). Four sections that revealed the most pulp structure (mid-root sections) were used for measurements and their means were used for statistical tests. Seven animals were analyzed for each experimental group and five for the control group.

The area for measurement on the alveolar bone was identified as a square parallel to the sagittal axis of the distobuccal root with a 200µm width and the length extending from the bifurcation to the end of the apex. Osteoclast surface was determined as the surface of an active osteoclast touching the alveolar bone and then divided by total bone surface per defined area.

Odontoclasts were considered as TRAP-positive multinucleated cells lying on the cementum or dentin and were counted on the mesial surface of the distobuccal root in the line starting from the bifurcation to the end of the apex of the distobuccal root. Odontoclast surface was considered as the surface of all odontoclasts divided by the total dentin surface that was extending from bifurcation to the end of the apex.

Von Kossa staining was performed using a NovaUltra™ von Kossa Stain Kit (IW-3014, IHC World, LLC., Woodstock, MD) according to the manufacturer's instructions.

To evaluate the two animals that had only either corticotomy or piezocision performed and sacrificed at day 4 and day 7, hematoxylin/eosin, TRAP and TUNEL stainings were performed. TRAP staining was used to evaluate the presence of osteoclasts at the surgical sites and TUNEL staining in order to evaluate the presence of cell death near the surgical sites to further explain the possible complications that were seen in the piezocision groups.

Paraffin sections were stained with the DeadEnd Fluorometric TUNEL System Kit (G3250, Promega Corp., Madison, WI) according to the manufacturer's instructions and analyzed using the Zeiss Axiovert 200 Microscope and digital camera. DAPI nuclear staining was performed to visualize total cells (counterstaining). The presence of apoptotic/necrotic cells was detected by using a FITC filter cube and nuclear labeling using a DAPI filter cube of the fluorescent microscope. The sections were evaluated on 5x, 10x and 20 x magnification.



## **K. Statistics**

Statistical analyses were carried out using GraphPad Prism (GraphPad Software Inc, La Jolla CA). Statistical significance of differences among means were determined by non parametric one way ANOVA test with a Tukey post-test. Significance was accorded when  $p < 0.05$ .

## **Chapter IV: Results**

During our experiments, 4 animals died during the procedures and were removed from the study. All 4 animals were lost due to accidental overdose of anesthetic. Three animals died at day 0 and one died at day 14. These animals were lost relatively early in the experiment and thereafter care was taken to carefully administer the amount of ketamine. Additional animals were added to the experiment to replace these animals in order to have equal numbers in each group.

During the last group of the experiment, one animal in the corticision with a full mucoperiosteal flap group was excluded from statistical analysis due to the spring debonding twice during the 21 days resulting in minimal tooth movement. The distance of tooth movement was extremely low compared to the rest of the animals in all groups and it was decided that this animal will be excluded from the group leaving only 13 animals in the corticision with a flap group as compared to 14 in the rest of the groups.

### **A. The Effect of Piezocision on Tooth Movement and the Surgical Complications**

Piezocision was performed on a total of 13 animals with 10 being in the Piezocision no flap group and 3 being in the Piezocision with flap group. These groups were cut short in number and not included in the statistical analysis due to numerous complications encountered after the surgical procedure and during the tooth movement process. The complications encountered were ulceration of the palatal mucosal where the procedure was performed, severe tipping of the first molar during traction, oro-antral communications adjacent to the distal surgical site, and exfoliation of the molars on day 21 of tooth movement (Figure 9). It was also observed that the animals with these complications did not gain weight at the same rate as animals who didn't have complications.

All animals displayed degrees of ulcerations on their palatal mucosa. Five animals exfoliated second molars at the end of tooth movement and 1 animal lost both second and third molar as well as part of its alveolus on day 21. One animal had a significant oral-antral communication adjacent to its second molar and its weight stayed lower than the rest of the group indicating that this animal was having difficulty eating with the injury.

MicroCT images were taken on these animals to evaluate the bone content around the first molar (Figure 10). From the 2-dimensional and 3-dimensional renditions it can be seen that there is a considerable resorption of the alveolus especially in between the first and second molars. This is what is contributing to the severe tipping and exfoliations of the molars and further evaluation was performed to attempt to determine if the bone was resorbing naturally or if it was a pathological process such as necrosis.

Three additional animals were added to the study to evaluate how the surgical injury progressed for 1 week post-surgery and start to uncover the pathophysiological process that is

occurring that caused these complications. The piezocision was performed and no tooth movement was done and then one animal was sacrificed on day 0, 4, and 7. Corticotomy was performed on the right side of the animal sacrificed at day 4 to compare with the piezocision on the contralateral side. The day 0 animal was to verify that the surgical procedure was penetrating the bone. Day 4 and 7 animals were analyzed histologically with H&E, TUNEL and TRAP staining. H&E staining demonstrated the types of cellular infiltrate around the surgical site (Figure 11). At day 4 there appeared to be more inflammatory cells including macrophages and polymorphonuclear leukocytes in the piezocision cut than the corticotomy cut. At day 7 the amount of inflammatory infiltrate decreased in the piezocision cut. TUNEL staining was performed (Figure 12) to evaluate if there was necrosis present at the surgical sites due to the ulcerations, resorption, and loss of teeth that occurred. TUNEL staining indicated that there was no necrosis present in either the day 4 or 7 animals. The TRAP staining (Figure 13) at day 4 showed no osteoclast infiltrate in the piezocision cut and a minimal number of osteoclasts in the corticotomy cut. At day 7 there were osteoclasts present in the piezocision group.

#### **B. The Effect of Corticotomy, Corticision with and without a Full Mucoperiosteal Flap on Tooth Movement**

To assess the effect of the surgical procedures on the rate of orthodontic tooth movement at a constant force, three methods of measurement were used. Feeler gauges were used to measure first molar mesialization and the distance between the first and second molar at day 4, 7, 11, 14, 17, and 21. PVS impressions were also taken intraorally at day 0, 7, 14, and 21 and die stone models fabricated to measure weekly rates of first molar movement. Lastly, microCT measurements from the height of contour of the first and second molar to measure the final amount of first molar mesialization at day 21 was performed.

For the Feeler gauge measurements at day 4, the mean maxillary left first molar mesialization among the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.31 \pm 0.05$  mm,  $0.44 \pm 0.04$  mm,  $0.37 \pm 0.06$  mm,  $0.35 \pm 0.05$  mm, respectively. An ANOVA comparison between groups at day 4 showed a statistically significant difference between groups ( $p < 0.0001$ ). A Tukey post hoc test (Figure 14) revealed that the amount of first molar mesialization recorded for the Corticotomy group was significantly more than the other groups. Also, mesialization in the Corticision no flap group was significantly greater than the OTM only group.

On day 7 the mean maxillary left first molar mesialization among the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.49 \pm 0.10$  mm,  $0.59 \pm 0.07$  mm,  $0.49 \pm 0.08$  mm,  $0.46 \pm 0.06$  mm, respectively. An ANOVA comparison between groups at day 7 showed a statistically significant difference between groups ( $p = 0.0005$ ). A Tukey post hoc test (Figure 14) revealed that the amount of first molar mesialization recorded for the corticotomy group was significantly more than the other groups.

On day 11 the mean maxillary left first molar mesialization among the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.66 \pm 0.14$  mm,  $0.80 \pm 0.10$  mm,  $0.70 \pm 0.07$  mm,  $0.62 \pm 0.12$  mm, respectively. An ANOVA comparison between groups at day 11 showed a statistically significant difference between groups ( $p = 0.0004$ ). A Tukey post hoc test (Figure 14) revealed that the amount of first molar mesialization recorded for the corticotomy group was significantly more than the OTM only and Corticision with flap groups.

On day 14 the mean maxillary left first molar mesialization among the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.83 \pm 0.13$  mm,  $0.96 \pm$

0.11 mm,  $0.89 \pm 0.09$  mm,  $0.77 \pm 0.14$  mm, respectively. An ANOVA comparison between groups at day 14 showed a statistically significant difference between groups ( $p=0.0007$ ). A Tukey post hoc test (Figure 14) revealed that the amount of first molar mesialization recorded for the corticotomy group was significantly more than the OTM only and Corticision with flap groups.

On day 17 the mean maxillary left first molar mesialization among the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $1.08 \pm 0.21$  mm,  $1.14 \pm 0.16$  mm,  $1.07 \pm 0.11$  mm,  $0.99 \pm 0.23$  mm, respectively. An ANOVA comparison between groups at day 17 (Figure 14) showed no statistically significant difference between groups ( $p=0.358$ ).

On day 21 the mean maxillary left first molar mesialization among the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $1.20 \pm 0.28$  mm,  $1.29 \pm 0.17$  mm,  $1.17 \pm 0.10$  mm,  $1.15 \pm 0.28$  mm, respectively. An ANOVA comparison between groups at day 21 (Figure 14) showed no statistically significant difference between groups ( $p=0.059$ ).

Stone models of the animal's maxillary left first, second, and third molars were measured on ImageJ for each group on day 0, 7, 14, and 21. Measurements were taken from the distal groove of first molar to the mesial groove of the second molar (Figure 6). The differences were taken between the days to illustrate the weekly rate of tooth movement from day 0-7, 0-14, 0-21, 7-14, 7-21 and 14-21 (Figure 15). Intraexaminer reliability was measured by taking measurements twice, more than two weeks apart, on four randomly selected models from each surgical group in each day. The intraclass correlation coefficient was 0.99 (95% confidence interval: 0.984-0.994) indicating excellent reliability in the measurement technique (Figure 16).

For the first week, day 0-7, the mean mesialization of the left maxillary first molar in the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.32 \pm 0.12$  mm,  $0.33 \pm 0.12$  mm,  $0.36 \pm 0.09$  mm,  $0.32 \pm 0.10$  mm, respectively. An ANOVA comparison (Figure 15) showed no significance difference between the groups ( $p=0.737$ ).

For the first two weeks, day 0-14, the mean mesialization of the left maxillary first molar in the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.68 \pm 0.24$  mm,  $0.75 \pm .17$  mm,  $0.73 \pm .16$  mm,  $0.67 \pm .23$  mm, respectively. An ANOVA comparison (Figure 15) showed no significance difference between the groups ( $p=0.675$ ).

For the 21 days of tooth movement, the mean mesialization of the left maxillary first molar in the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $1.17 \pm 0.31$  mm,  $1.30 \pm 0.30$  mm,  $1.11 \pm 0.18$  mm,  $1.08 \pm 0.47$  mm, respectively. An ANOVA comparison (Figure 15) showed no significance difference between the groups ( $p=0.3337$ ).

For the second week of tooth movement, day 7-14, the mean mesialization of the left maxillary first molar in the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.37 \pm 0.17$  mm,  $0.42 \pm 0.11$  mm,  $0.37 \pm 0.13$  mm,  $0.35 \pm 0.19$  mm, respectively. An ANOVA comparison (Figure 15) showed no significance difference between the groups ( $p=0.667$ ).

For the third week, day 14-21, the mean mesialization of the left maxillary first molar in the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.49 \pm 0.15$  mm,  $0.55 \pm 0.18$  mm,  $0.38 \pm 0.13$  mm,  $0.42 \pm 0.30$  mm, respectively. An ANOVA comparison (Figure 15) showed no significance difference between the groups ( $p=0.129$ ).

For the last two weeks, day 7-21, the mean mesialization of the left maxillary first molar in the OTM only, Corticotomy, Corticision no flap, and Corticision with flap groups were  $0.85 \pm$

0.24 mm,  $0.96 \pm 0.24$  mm,  $0.75 \pm 0.16$  mm,  $0.77 \pm 0.45$  mm, respectively. An ANOVA comparison (Figure 15) showed no significance difference between the groups ( $p=0.199$ ).

An ANOVA comparison was also performed to evaluate if there was a significant difference in tooth movement from week to week. The comparison showed a statistically significant difference between groups ( $p=0.003$ ). A Tukey post hoc test (Figure 17) revealed that the amount of first molar mesialization recorded for the third week (day 14-21) Corticotomy group was significantly greater than the first week of OTM only group, 1<sup>st</sup> week of Corticotomy group, and the first and second week of the Corticision with flap group.

At the end of the 21 days all animals were sacrificed and the maxillae hemisected and cleansed of soft tissue. The hemisected maxillae were then submitted for microCT analysis and maxillary molar mesialization was evaluated by measuring the distance between the distal height of contour of the maxillary first molar and the mesial height of contour of the maxillary second molar (Figure 18).

MicroCT analysis illustrated that for OTM only, Corticotomy, Corticision no flap and Corticision with flap groups the mean amount of maxillary first molar mesialization was  $0.98 \pm 0.24$  mm,  $1.12 \pm 0.22$  mm,  $0.93 \pm 0.21$  mm, and  $1.02 \pm 0.50$  mm, respectively. To evaluate the statistical differences in the amounts of tooth movement between groups an ANOVA comparison was performed (Figure 18). The results of this test found that there was no significant differences in the final amounts of the tooth movement observed after day 21 ( $p=0.703$ ).

Since three different measurement techniques were used to evaluate that final distance of first molar mesialization at Day 21, an ANOVA comparison was performed to evaluate if there was any significant difference between the measurement methods to provide insight on how accurate these methods were in capturing the correct distances. The ANOVA indicated that

there was no significant difference between measurements in both the OTM only ( $p=0.0788$ ) and Corticision with flap groups ( $p=0.747$ ). There were significant differences in measurements in both the Corticotomy ( $p=0.0251$ ) and Corticision no flap group ( $p=0.0016$ ) (Figure 19). A Tukey post hoc test revealed that the microCT measurements were significantly less than the model measurements in the Corticotomy group and less than both model and feeler gauge measurements in the Corticision no flap group.

### **C. The Effect of Corticotomy, Corticision With and Without a Flap on the Alveolus**

To evaluate the effect of tooth movement and surgical procedures on the alveolus microCT images of the maxillary first molars were obtained for analysis. A non-treated control group of five animals was also analyzed along with the four surgical groups for comparison.

Tissue density was calculated from the microCT scans and the mean values for Control, OTM only, Corticotomy, Corticision no Flap, and Corticision with flap groups were  $1085 \pm 14$  mm/ccm HA,  $1043 \pm 15$  mm/ccm HA,  $1059 \pm 58$  mm/ccm HA,  $1046 \pm 37$  mm/ccm HA,  $1037 \pm 47$  mm/ccm HA, respectively. An ANOVA comparison was performed (Figure 121) and there was no significant difference between the groups ( $p=0.137$ ).

Bone Volume Fraction was calculated from the microCT scans and the mean values for Control, OTM only, Corticotomy, Corticision no flap and Corticision with flap were  $78.0 \pm 8.4\%$ ,  $78.2 \pm 5.1\%$ ,  $78.4 \pm 14.1\%$ ,  $80.3 \pm 6.8\%$ , and  $80.0 \pm 8.3\%$ , respectively. To compare all groups an ANOVA comparison was performed (Figure 21). This indicated that there was no significant difference between the groups ( $p=0.177$ ).



#### **D. The Effect of the Surgical Procedures on Osteoclasts**

Osteoclast activity was investigated by counting osteoclasts and calculating osteoclast surface area histologically in all groups. Osteoclast numbers were the highest in the Corticotomy group ( $4.25 \pm 3.21$ ) followed by OTM only ( $3.71 \pm 4.07$ ), Corticision with flap ( $3.18 \pm 2.02$ ), Corticision without a flap ( $2.27 \pm 1.37$ ), and control ( $0.375 \pm 0.411$ ) groups. However, when all groups were compared (Figure 23) using ANOVA, the differences observed were found to be non-significant ( $p=0.1321$ ). Osteoclast surface area measurements were the largest in the Corticotomy group ( $0.077 \pm 0.0588$  per mm) followed by OTM only ( $0.041 \pm 0.071$  per mm), Corticision with flap ( $0.0355 \pm 0.021$  per mm), Corticision without a flap ( $0.0295 \pm 0.019$  per mm), and control ( $0.0049 \pm 0.0078$  per mm) groups. However, these measurements were non-significant when compared with ANOVA ( $p=0.1062$ ) (Figure 24).

#### **E. The Effect of the Surgical Procedures on Odontoclasts**

Odontoclast activity was measured by counting odontoclasts and calculating odontoclast surface area on the mesial of the distobuccal root by histology in all groups. This was performed in order to correlate root resorption with odontoclast activity. Odontoclast number was highest in the Corticotomy group ( $2.27 \pm 1.48$ ) followed by OTM only ( $1.44 \pm 1.50$ ), Corticision with flap ( $1.07 \pm 0.76$ ), Corticision no flap ( $0.76 \pm 0.80$ ), and control ( $0.00 \pm 0.00$ ) groups. When all groups were compared (Figure 25) using ANOVA, the differences observed were found to be significant ( $p=0.0182$ ). A Tukey post hoc test revealed that the Corticotomy group had significantly more odontoclasts than the control group.

Odontoclast surface area was measured and had a similar trend to odontoclast number. The Corticotomy group demonstrated the highest surface area ( $7.91 \pm 5.12$  %). This was followed by OTM only ( $4.39 \pm 4.10$  %), Corticision with flap ( $2.92 \pm 1.75$  %), Corticision no

flap ( $2.45 \pm 2.50 \%$ ), and control ( $0.00 \pm 0.00 \%$ ) groups. When all groups were compared using ANOVA (Figure 26), the differences observed were found to be significant ( $p=0.005$ ). Tukey post hoc test revealed that the Corticotomy group had significantly more surface area than the Corticision no flap and control groups.

#### **F. The Effect of the Surgical Procedures on Root Resorption**

The effect of tooth movement and surgical procedures on the root surface was studied using the microCT scans. Root resorption was calculated from the surface of the distobuccal root in the control group and all four surgical groups and reported as a Root Volume Fraction (RVF). The mean RVF for the control, OTM only, Corticotomy, Corticision no flap and Corticision with flap were  $63.0 \pm 1.1\%$ ,  $58.8 \pm 3.6\%$ ,  $58.9 \pm 2.7\%$ ,  $55.0 \pm 2.9\%$ , and  $59.0 \pm 3.3\%$ , respectively. An ANOVA comparison was performed (Figure 27) and it showed that there was a significant difference between the groups ( $p=0.0258$ ). A Tukey post hoc test revealed that there was significantly more resorption in the Corticision no flap group as compared to the control group.

## **Chapter V: Discussion**

The average length of orthodontic treatment has been shown to be between 21 and 35 months depending on complexity and extraction needs of the patient (1, 2). As orthodontic treatment increases in time, there is a proportional increase in unwanted side effects such as caries, periodontal bone loss, root resorption, white spot lesions, and decreased patient cooperation (4, 5, 7, 71). Patients have also expressed an interest to decrease the amount of time they are wearing braces. Decreasing the treatment time can thus be beneficial for both the patient and the orthodontist due to a decrease in unwanted side effects as well as increased patient satisfaction.

To decrease treatment time, orthodontists must increase the rate of orthodontic tooth movement. The rate at which teeth move through the alveolus is dependent on the biological processes occurring in the surrounding bone as force is applied to the individual teeth. These rate limiting steps include bone turnover, bone density, and the degree of hyalinization of the PDL (8). Therefore, the current research and modalities to increase tooth movement would need to target these processes to decrease treatment time.

Techniques to modulate these biological processes can be organized under the categories of pharmacological, non-invasive mechanical, and adjunctive surgical modalities.

Pharmacological interventions have shown the ability to increase tooth movement but not without side effects, like hyperalgesia, severe root resorption and other drug induced systemic and local side effects (19). Although showing future potential, much research is needed to understand the complexity of these pharmacological reactions. This has led to an increased interest in other physical methods such as mechanical vibration. Vibration is a non-invasive technique that companies, like AcceleDent<sup>TM</sup>, have claimed to increase tooth movement without

substantial research backing up their claims. In fact, research has recently shown that mechanical vibration can actually inhibit tooth movement (21). Thus, the most promising field to increase the rate of OTM is the implementation of surgical methods.

Surgical modalities take advantage of the regional acceleratory phenomenon (RAP) proposed by Frost in 1983. This phenomenon is based on the acceleration of bone healing and turnover properties in response to a noxious stimulus to the bone. The stimulus, when directed at bone, can accelerate bone turnover and reduce regional bone density leading to a transient state of osteopenia. The stimulus and RAP have been said to be proportional, that is, the larger the noxious stimulus the greater the RAP. If RAP could be induced in the alveolus around the teeth then, theoretically, the bone could turnover at a higher rate leading to teeth moving faster through the surrounding bone (30, 31). Three surgical procedures have been shown in the literature as common techniques used to induce RAP and increase OTM; these include Corticotomy, Corticision, and Piezocision.

Made popular by Wilcko and Wilcko, corticotomy involves a full mucoperiosteal flap and fissures made through the buccal and/or lingual plates that surround the bone (45). Corticision, popularized by Kim and Park (26, 51), uses a reinforced scalpel as a thin chisel to separate the interproximal cortices without raising a full flap. The piezotome, an instrument widely used in neurosurgery and periodontics, uses a blade and ultrasonic micro-vibrations to cut bone through small incisions made in the soft tissue (57). Studies have shown that all of these can be efficient means of stimulating tooth movement by inducing RAP and accelerating bone remodeling (27, 45, 51). However, each of these procedures involves a different level of invasiveness, whether it is raising a full mucoperiosteal flap or not or traumatizing the bone. The different levels of invasiveness could lead to different RAP responses and levels of tooth

movement. Another factor to consider when moving teeth is that this reaction is transient meaning that the procedure might be needed to be performed again to maintain the rate of OTM for the total movement to occur. A more invasive procedure would make a patient less willing to undergo the surgery multiple times. It is important to find which technique produces the most efficient tooth movement while considering whether patients would agree to multiple procedures should the need for it arise. Thus, the purpose of this study was to assess the effect of Corticotomy, Corticision, and Piezocision with and without a full mucoperiosteal flap on the amount of tooth movement and changes in the alveolus in a rat model when a constant force was applied for 21 days.

As the experiments progressed and piezocision was performed many significant complications were encountered. These included oro-antral communications, ulcerations of the palatal mucosa where the procedure was performed, severe tipping of the first molars, and exfoliation of the first and second molars toward the end of the 21 days (Figure 10). It was also observed that the animals with complications did not gain weight at the same rate as animals who didn't have complications. This could have significant effects on bone metabolism and rate of tooth movement, thus providing another reason to omit these animals from statistical analysis.

One hypothesis that could be contributing to these complications is that the device used was intended for use on humans. Thus the bone cutting settings were set for a larger animal with frequency of ultrasonic microvibration too powerful for a rat specimen. This could be a possibility because all reports in the literature have shown excellent and fast healing in humans who have had piezocision performed (59). Another hypothesis is that it was very difficult to provide adequate irrigation to the surgical site due to both the size of the animal and inadequate equipment to suction the irrigation out of the animal's mouth. Having little irrigation could

cause overheating of the bone and surrounding soft tissue inducing necrosis and causing the aforementioned complications. In a recent review article by Yaman et al. in 2013, a description of the proper handpiece movement when performing cuts was described. It was suggested that the handpiece be moved backward and forward continuously at a high speed with minimum pressure as opposed to slow movements and excessive pressure (72). The latter decreases the micro-movements of the blade and causes an increase in bone temperature. In the present study, the smallest blade that could be used with the device was two millimeters, which was the exact length of the cut that was needed. The cut was performed by applying straight vertical pressure to sink the blade into the cortical bone approximately one millimeter. The suggested movement of high speed back and forth motion with minimum pressure could not be applied due to the size of the blade and surgical area. This could have contributed to the increase in temperature of the bone and necrosis leading to the ulcerations, excessive resorption and severe tipping. This further suggests that this device made for human surgical procedures might not be suitable for rats. Lastly, most of these complications were seen between the first and second molar which was where the distal cut was performed. This area of bone on the palate is very small and the nasal cavity can easily be penetrated with inaccurate positioning and depth of the piezosurgical blade, leading to the potential of excess damage and oral-antral communication.

MicroCT images of the animals that had complications (Figure 10) demonstrate significant resorption or loss of bone between the first and second molar. This loss of bone could either be resorption occurring from the RAP process or necrosis from damaging the bone with the surgical blade. In order to investigate the cause of the complications, alterations were made in the study to two animals. Instead of continuing tooth movement studies in the Piezocision with and without flap groups, one animal had piezocision and corticotomy performed with no tooth

movement and was sacrificed at day 4 and one animal received just piezocision with no tooth movement and was sacrificed at day 7. This was done to begin to evaluate the pathophysiology responsible for these complications and compare them to corticotomy procedures. Hematoxylin & Eosin (H&E) staining, TUNEL assay, and TRAP staining were performed to evaluate the surgical sites. H&E staining demonstrated (Figure 11) that at day 4 there appeared to be more inflammatory infiltrate in the piezocision cut than the corticotomy cut. This infiltrate decreased by day 7. The TRAP staining (Figure 13) at day 4 showed no osteoclast infiltrate in the piezocision cut and a minimal amount of osteoclasts in the corticotomy cut. At day 7 there were osteoclasts present in the piezocision group. This all coincides with the cellular recruitment process in bone turnover where early inflammatory infiltrate is observed. The infiltrate is a precursor or recruiter for the osteoclasts that are seen at day 7 (73). These osteoclasts could be contributing to the excessive resorption and tipping seen in the piezocision animals.

TUNEL staining was performed (Figure 12) to evaluate if there was necrosis present at the surgical sites due to the ulcerations, resorption, and loss of teeth. TUNEL indicated that there was no necrosis present in either the Day 4 or 7 animals. This demonstrates that, if performed properly, piezocision can set up an environment where osteoclasts are recruited without damaging the surrounding tissue and establish a RAP process. Research needs to be continued in this area to refine the piezocision technique in rats and uncover the underlying pathophysiological mechanism that caused these complications.

Tooth movement was evaluated using three techniques: feeler gauge, stone models, and microCT. Three techniques were used for several reasons. MicroCT only evaluates the final amount of tooth movement after the animals are sacrificed at day 21. Feeler gauge and stone models were used to quantify and compare weekly tooth movement throughout the experiment.

Also, previous studies at the University of Connecticut Orthodontic Department (53, 54), have used feeler gauges, and for comparison purposes, it was decided to use them again. However, the feeler gauge method's accuracy came into question in these studies, thus the reason for using stone models to further evaluate weekly tooth movement between the groups.

Feeler gauge measurements (Figure 14) indicated a significant difference among groups at day 4, 7, 11, and 14 ( $p < 0.0001$ ,  $p = 0.0005$ ,  $p = 0.0004$ ,  $p = 0.0007$ ). At these days, it was observed that the corticotomy group had significantly greater tooth movement than the other groups. However, as time progressed, groups began to become more similar, where at day 17 and day 21, the differences in measurements between the groups were insignificant. This may indicate that the surgical procedures have an initial transient effect which eventually dissipates after two weeks, further suggesting the need for more than one surgical procedure during treatment. This was a similar result seen in previous studies by Murphy et al. (54) using feeler gauges. Stone model measurements (Figure 15) did not show similar trends and could not corroborate the feeler gauge findings in terms of weekly tooth movement. All weekly stone model measurements were insignificant when comparing groups. With the stone model measurements, weekly tooth movement measurements were compared to observe differences in the rate of tooth movement from week to week. This was performed due to the transient effect of these procedures and previous research showing different rates of weekly tooth movement (26, 34). The data (Figure 17) demonstrated that there was a significant difference between the groups' weekly measurements. The third week of the Corticotomy group tooth movement was greater than the first week of OTM only, Corticotomy and Corticision with flap groups. This trend and the observation that tooth movement was greater in all groups during the third week was very similar to the piezocision results of Dibart et al. (60).



MicroCT measured only the final amount of tooth movement after the animals were sacrificed at day 21 (Figure 18). No significant difference was observed between groups at the conclusion of the 21 days. Similarly, there was no significant difference across measurement techniques. These findings all support the null hypothesis of no difference in the amount of tooth movement between the groups.

One trend that was seen but couldn't be verified by statistics was consistently greater tooth movement in the corticotomy group. In every figure it can be seen that corticotomy had greater weekly and final measurements throughout the experiment, but due to the large variability in all groups everything was insignificant. This is in line with the theory that the more invasive and noxious the stimuli, the larger the RAP process causing a greater amount of tooth movement. The position of our corticotomy holes 5mm mesial to the first molar (Figure 3) might contribute to this not being significant and the RAP process not occurring close enough to the tooth to increase bone turnover around the teeth. It is suggested for future studies that the injury be closer and even around the first molar to create a RAP process around the tooth.

Alveolar changes were evaluated using microCT and histomorphometry analyses. MicroCT analysis was used to study the effects of the surgical procedures on the alveolus at the furcation of the maxillary first molars. Parameters studied included bone volume fraction and tissue density. Both parameters were not significant when all groups were compared (Figure 21). This correlated with the insignificant tooth movement between the groups at day 21. This was similar to the previous studies on corticision without a flap (54). One interesting finding was that the control group was also not significant compared to all groups. It was thought that tooth movement, with or without a surgical procedure, would lead to a decrease in the BVF and tissue density due to an increase in bone turnover and the RAP process.

Osteoclast number and osteoclast surface area were measured (Figure 23 & 24) to correlate presence of osteoclasts with the RAP process, increased bone turnover, and increased tooth movement. There was no significant difference in number and surface area which correlates with the insignificant difference in tooth movement between groups. There was a similar trend in tooth movement, osteoclast number, and surface area where the greater the tooth movement the greater the osteoclast number and surface area.

To study the effect of these three surgical procedures on the root surface of the first molar, the distobuccal root was evaluated for changes using microCT analysis. When all groups were compared including a control group with no tooth movement, there was a significant difference between the control group and the Corticision no flap group (Figure 27). There was more resorption in the Corticision no flap group. There was no significant difference between Corticotomy and Corticision with flap groups and the control. These two groups require a flap, so this could indicate a protective effect against root resorption since a flap being raised can cause an increase in RAP. Previous studies have not shown that there is a possible protective effect of surgical procedures on root resorption (74).

Odontoclast number and surface area on the distobuccal root were also calculated (Figure 25 & 26) to correlate with root resorption. The groups with greater root resorption should have a greater number of odontoclasts and larger surface area on the root since odontoclasts cause root resorption. However, this was not demonstrated with our samples. Corticotomy, which had an insignificant amount of root resorption compared to the other groups, had a significantly greater number of odontoclasts and larger surface area of odontoclasts on the root when compared to the control and Corticision no flap group.

An objective of this study was to observe the effects of a full mucoperiosteal flap on the rate of orthodontic tooth movement. Significant resorption of the alveolus was seen in studies by Yaffe et al. when flaps were raised on the buccal and lingual surfaces demonstrating an alveolar response to separating the periosteum from the bone (32). Swapp et al. suggested that elevating a flap could contribute to the RAP response when they observed no significant difference in tooth movement in flapless surgical procedures compared to the control (61). The present study consisted of a Corticotomy group which traditionally requires a flap and a Corticision and Piezocision group which do not require flaps and are less invasive. Corticision and Piezocision with flap groups were added to compare the flaps effect. It was shown that there was no significant difference in tooth movement or alveolar response. This does not support the hypotheses by Swapp et al. (61), but it supports our null hypothesis that a flap does not have an effect on tooth movement or alveolar response. This suggests that alveolar bone damage may need to be more extensive and consist of damaging the medullary bone beneath the cortical bone to induce more of a response. Kim et al. used corticision without a flap in a feline model and the surgical blade was driven to depths of up to 10mm into the bone, providing further damage to the underlying trabecular bone (26). They observed a significant increase in tooth movement suggesting our procedures need to be deeper in the bone.

This study did have its limitations. The limitations were in both the experimental and evaluation methods and could help explain some of the findings. There was a considerable amount of variation between animals in the same surgical groups. This could be due to variability between animals and differences in the placement and activation of the spring. The groups of animals that were received were not necessarily from the same parents which could cause variability in growth and bone metabolism between the animals. Since the experiments

started when the animals were six weeks old, they went through considerable growth throughout the three weeks of tooth movement. This growth could affect the spring placement and activation as well as bone metabolism and turnover. For future studies it is recommended that an older animal be used. Rats grow continuously throughout life, although at a decreasing rate when they are older. This will help standardize the experiment and will provide a larger area to work for better spring placement.

Furthermore, on day 7 and 14 the springs required re-activation to ensure they were activated throughout the 21 days to compensate for the eruption of the incisors and mesial movement of the first molar. Based on previous studies of spring characteristics (Figure 1) (Murphy), it is known that the springs would provide desired amounts of force within a specific window of activation. When activated approximately one millimeter, the springs in this experiment provided ~10g of force. But if the springs were activated beyond one mm an additional 14-15 grams of force was applied per millimeter. Every animal varied in size and the distance between the first molar and incisors were different as well. Also, the incisors grew at different rates and the molars mesialized at different amounts which all contribute to different activation lengths of the springs providing different forces to the molars. A standardized procedure of checking the activation of the spring and force delivery would help standardize the force placement on the first molar and possibly decrease the variability seen between animals.

As mentioned before, there was no significant difference between our three measurement techniques but it was felt that there were shortcomings to the evaluation methods of feeler gauge and stone model measurements. For the feeler gauge measurements, visualization was difficult and thus it was difficult to determine the fit of the gauge between the molars. Also, when molars begin moving, there is an increase in mobility. Therefore, when the gauge is placed with poor

visibility the molars can be inadvertently pushed apart, causing larger measurements. Even though not significant, the trend of larger tooth movement measurements can be seen in the feeler gauge group (Figure 19).

The stone models were fabricated to provide an alternative method to the feeler gauge for weekly tooth movement measurements. PVS impressions and stone models provide an extremely accurate representation of the teeth but inaccuracy occurs when taking photographs of the models. Photographs were taken parallel to the occlusal plane but change in angulation of the camera, or the model, away from parallel could change the measurements considerably from model to model. Performing sub-millimeter measurements could be very inaccurate depending on the angulation.

Lastly, due to the method of force application and molar mesialization applied, with the springs attached at the level of the cemento-enamel junction above the center of resistance, the teeth tended to tip mesially and rotate palatally. This type of movement affects our analysis of the alveolar changes and root resorption. It was assumed that the mesial surface of the distobuccal root represented an area of compression, however, due to the tipping and rotation, there were actually areas of both compression and tension on the root analyzed. Also, evaluation of root resorption changes was performed on the distobuccal root. Since the RAP process took place on the mesial aspect of the first molar, this could have been too distant from the distobuccal root for it to have any response to the procedure.

## **Chapter VI: Conclusions**

The main objective of this study was to evaluate three different surgical procedures and the impact of a full mucoperiosteal flap on the rate of orthodontic tooth movement. Feeler gauge measurements demonstrated a significant difference in the amount of tooth movement between groups at day 4, 7, 11, and 14. At day 4 and 7, the corticotomy group showed significantly greater tooth movement than all other groups. At day 11 and 14 the corticotomy group showed significantly greater tooth movement than just the OTM only and Corticision without a flap group. At day 17 and 21 there was no significant difference in the amount of tooth movement between groups. Stone model measurements did not agree with day 7 and day 14 feeler gauge measurements and there was no significant difference between groups. At day 21, feeler gauge, stone model measurements agreed that there was no significant difference in tooth movement between the groups. Based on these findings the null hypothesis of no difference in the amount of tooth movement among the experimental groups must be accepted.

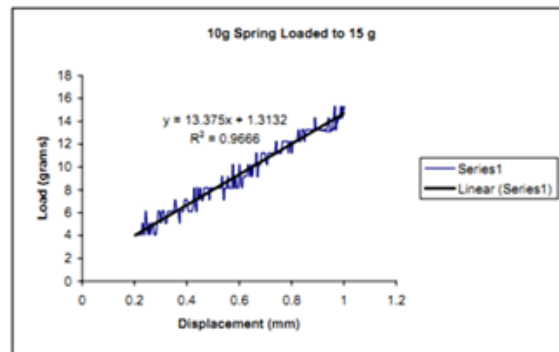
Corticotomy, Corticision with and without a flap and their effect on the alveolus was studied histologically and using microCT. At the end of tooth movement on day 21 there was no significant difference in tissue density and BVF when compared to OTM only groups or a no tooth movement control group. Histomorphometric analysis indicated that there was no significant difference in osteoclast number or surface area which correlated with tooth movement and supported the null hypothesis.

The surgical procedure's effect on root resorption was evaluated by microCT analysis. This analysis showed that there was a significant difference between groups and the Corticision no flap group had more root resorption than all other groups. This could indicate a protective effect of raising a flap on root resorption.

The piezocision group experienced a number of complications that caused them to be excluded from statistical analysis. The complications included oral-antral communications, ulcerations of the palatal mucosa where the procedure was performed, severe tipping of the first molars, and loss of first and second molars toward the end of the 21 days. Preliminary analysis of the surgical sites at days 4 and 7 showed a significant amount of inflammatory infiltrate and osteoclasts that could be contributing to the resorption and tipping. This promising area of the study will need to be studied more in depth and the surgical technique will need to be refined in order to evaluate physiological tooth movement in this model.

In conclusion, on day 21, Corticotomy and Corticision with and without a full mucoperiosteal flap groups do not show a statistically significant difference for OTM and alveolar response compared to the OTM only group or between experimental groups. Furthermore, studies involving piezocision will need to be continued to uncover the underlying pathophysiology and surgical technique errors that were occurring in this experiment.

## Chapter VII: Figures



**Figure 1:** Spring stress strain curve for approximately 10 nickel titanium coil springs

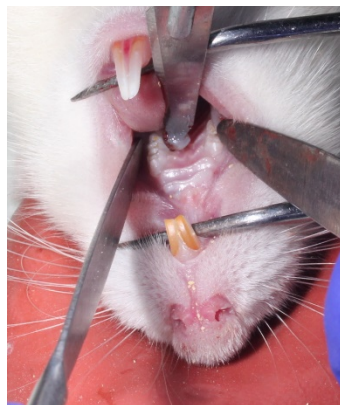


**Figure 2: Spring Placement:** 10g nickel titanium spring from the left maxillary first molar to the maxillary central incisors

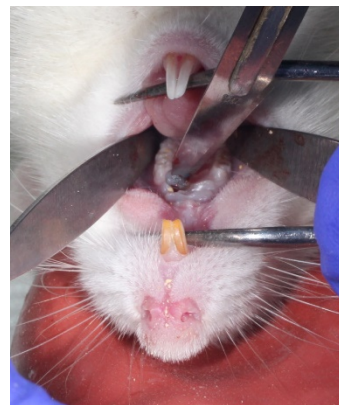




**Figure 3: Corticotomy Procedure:** Corticotomy with a full thickness mucoperiosteal flap and three .25 x .25mm holes were placed in the cortical bone with a 1/4 round bur mesial to the left maxillary first molar.

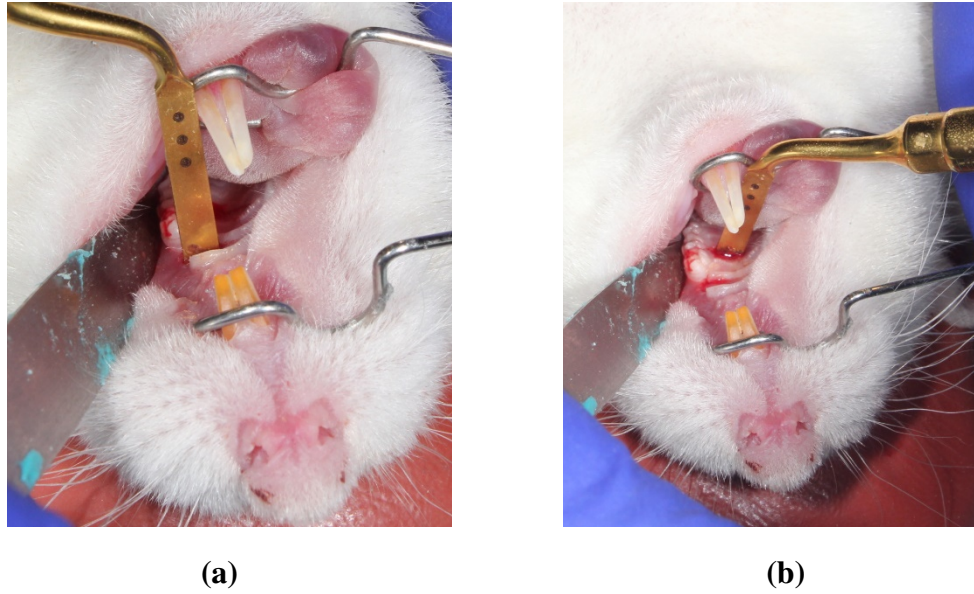


(a)

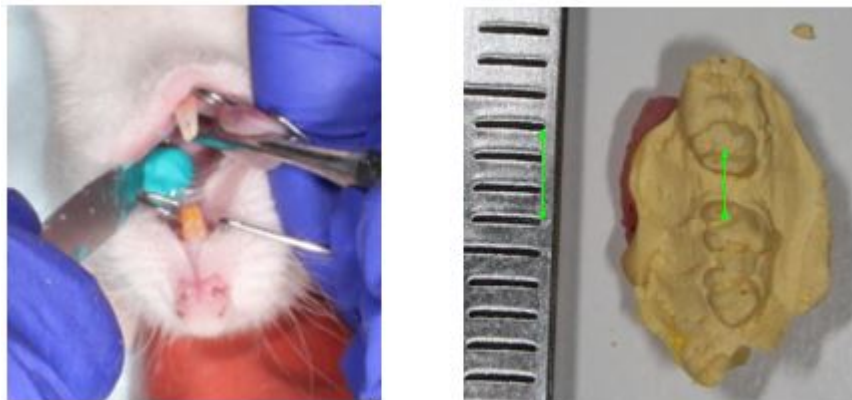


(b)

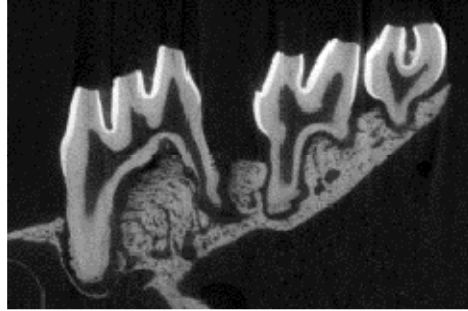
**Figure 4: Corticision Procedure:** a) Corticision without a full mucoperiosteal flap procedure with the No.11 Bard-Parker blade being inserted into the mesial-palatal gingiva adjacent to the left maxillary first molar. b) Same procedure on the disto-palatal gingiva adjacent to the left maxillary first molar. Note the composite stop on the blade to control the depth of the cut into the cortical bone.



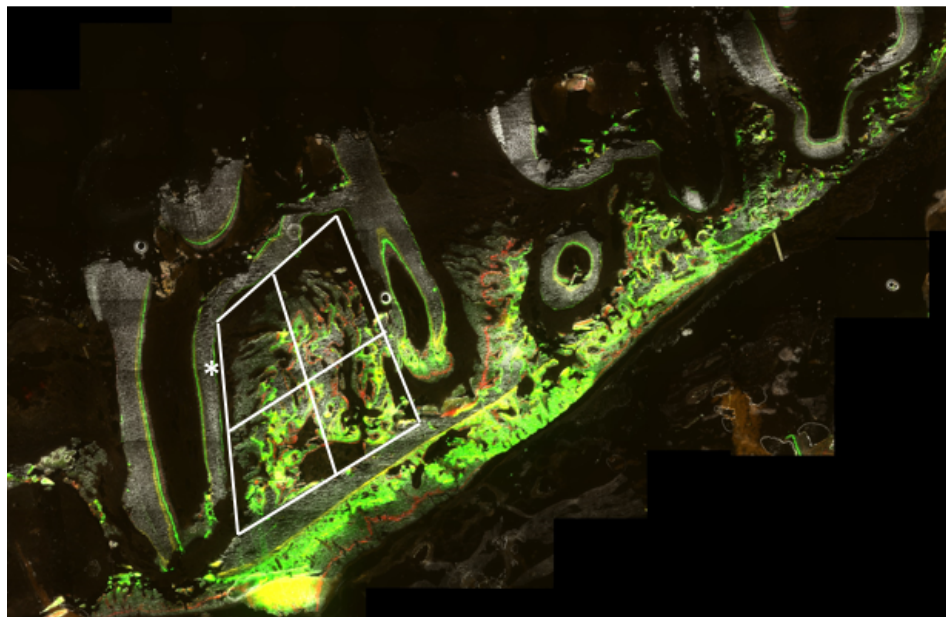
**Figure 5: Piezocision Procedure:** a) Piezocision without a full mucoperiosteal flap procedure with a Piezosurgery insert of 2mm being inserted into the mesial-palatal gingiva adjacent to the left maxillary first molar. b) Same procedure on the disto-palatal gingiva adjacent to the left maxillary first molar.



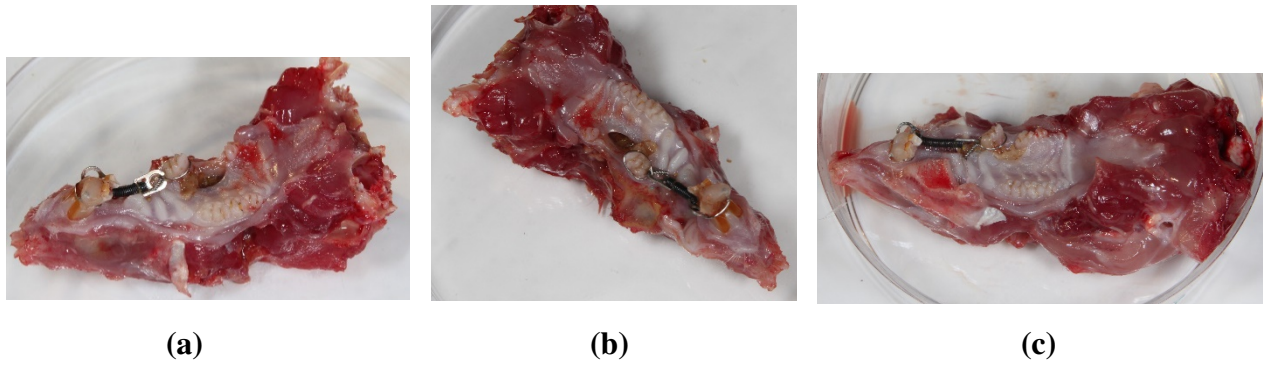
**Figure 6: PVS Impression and Stone model Measurement:** PVS impressions were taken at Day 0, 7, 14, and 21 days and dental die stone models were fabricated and measured adjacent to a millimeter ruler to calculate weekly, biweekly and final tooth movements.



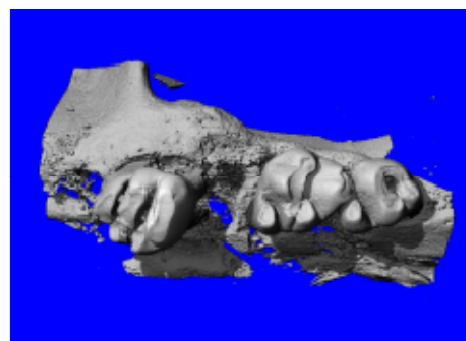
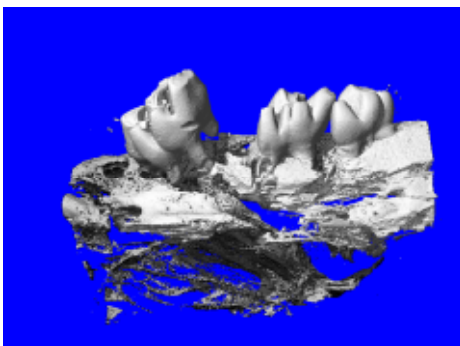
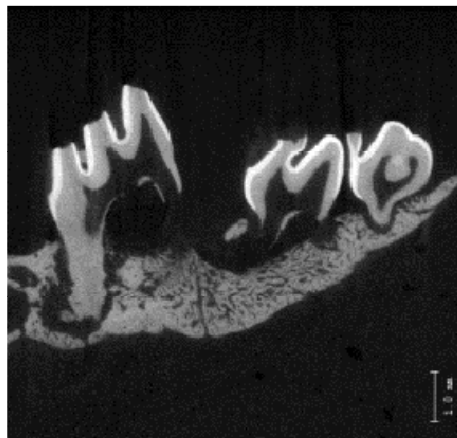
**Figure 7: Micro-CT image at day 21.** Tooth movement and bone parameter measurements were taken from the images.



**Figure 8: Fluorescent Imaging.** For quantitative analysis of bone labeling, the alveolar bone in furcation area was divided into 4 equal quadrants in the sagittal plane and only the coronal quadrant adjacent to the mesial root was considered to be a bone forming area or region of interest.



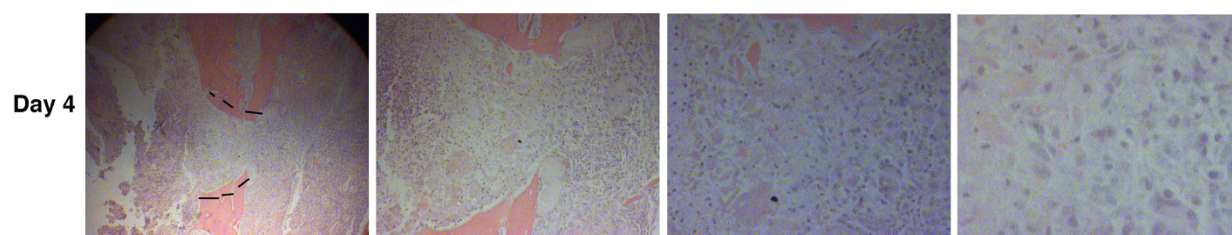
**Figure 9: Complication with the Piezocision surgical procedure at day 21.** (a) and (b) The same dissected maxilla from the Piezocision without a flap group illustrating the oral-antral communication, ulceration, and loss of the second molar. (c) Dissected maxilla from the Piezocision with flap group showing ulceration of the palatal mucosa and severe tipping of the first molar.



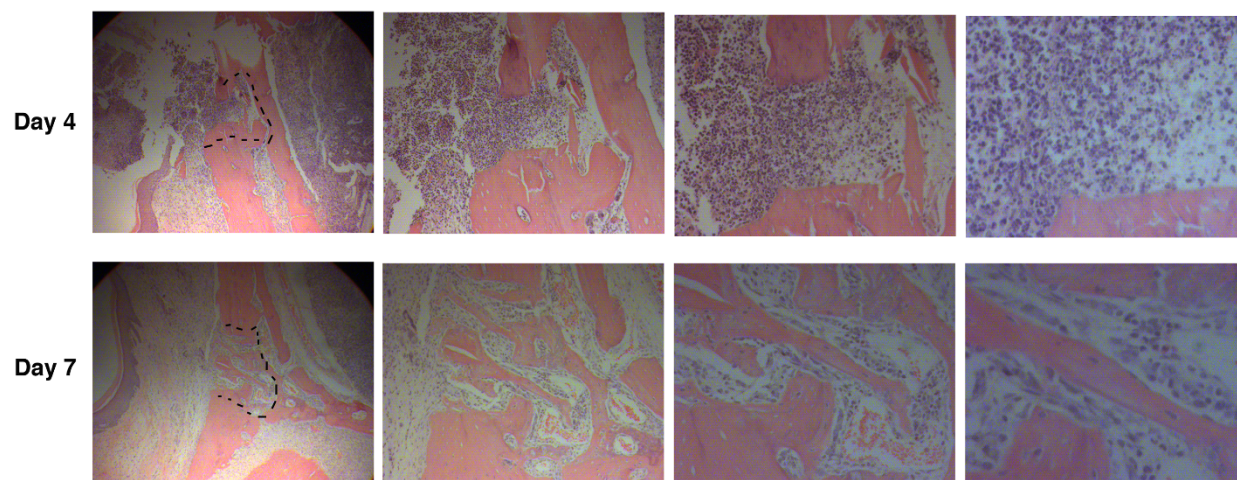
**Figure 10: MicroCT images of Piezocision group at Day 21.** Sagittal and Coronal views of 2D and 3D renditions of a Piezocision with no flap group. There is a significant amount of resorption between the 1<sup>st</sup> and 2<sup>nd</sup> molars and excessive tipping of the first molar in response to the orthodontic force.



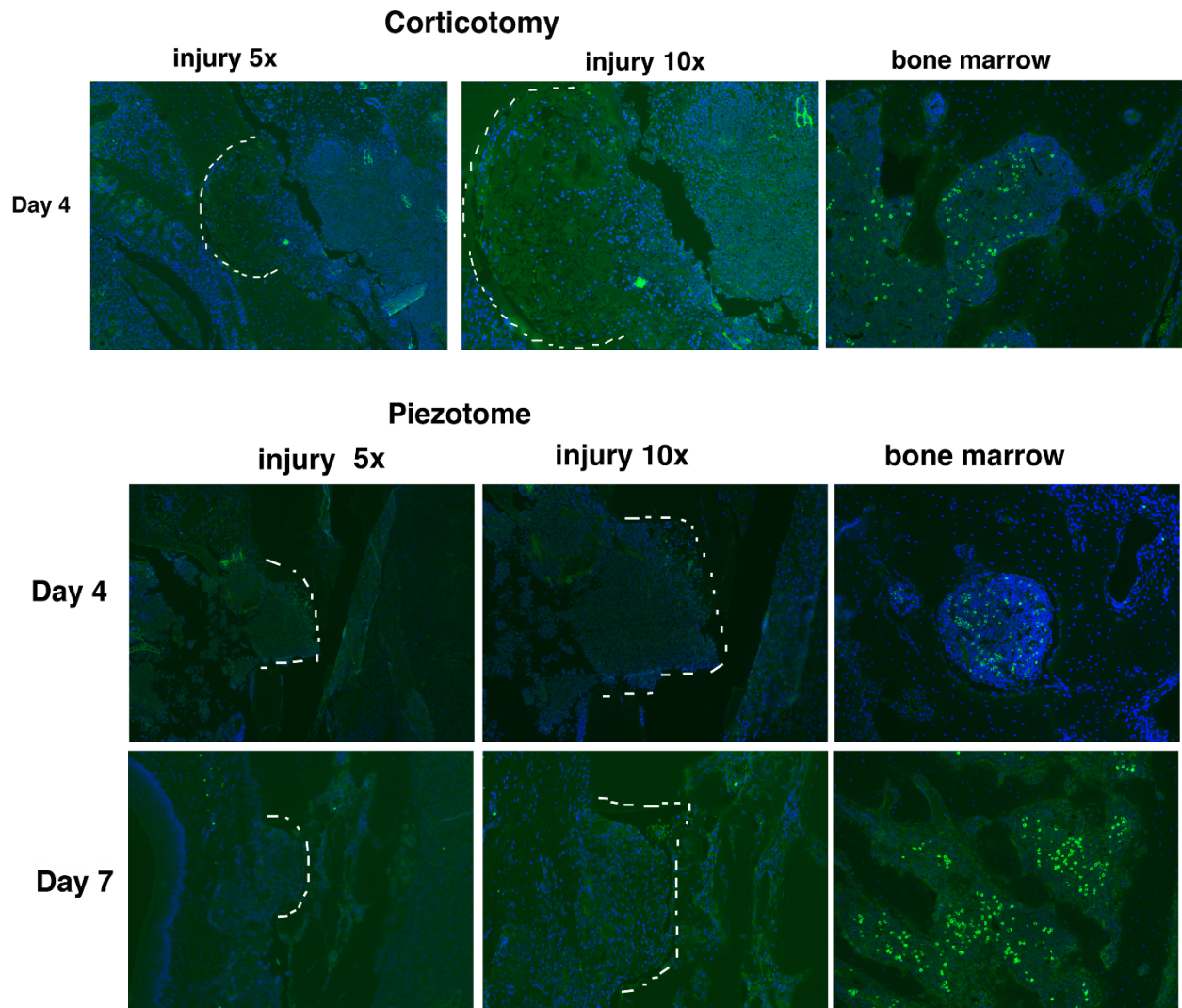
### Corticotomy



### Piezotome



**Figure 11: Hematoxylin and eosin staining.** Day 4 for the corticotomy group shows less inflammatory infiltrate than the Day 4 piezocision group. Day 7 for the piezocision group shows much less inflammatory infiltrate than Day 4.

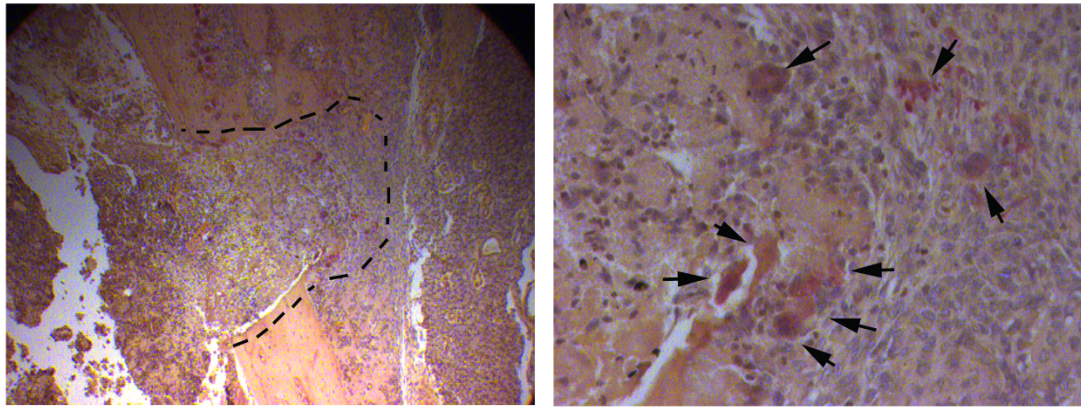


**Figure 12: TUNEL assays.** TUNEL staining was performed to examine if there was necrosis present from the surgical injury to help explain the complication seen. No necrosis is present at Day 4 or 7 in the corticotomy and piezocision groups.



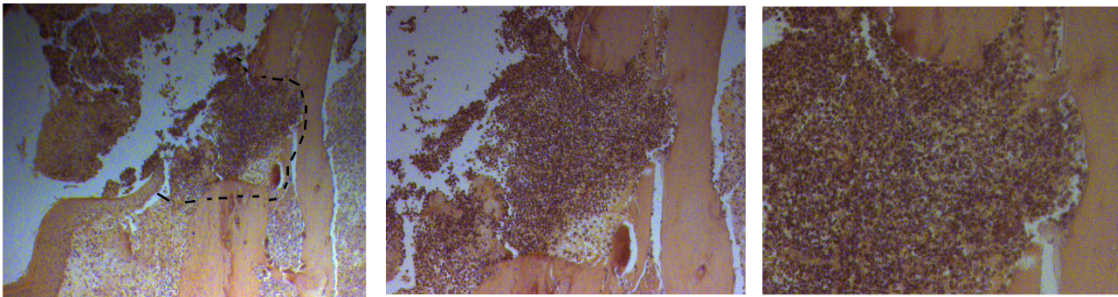
## Corticotomy

Day 4

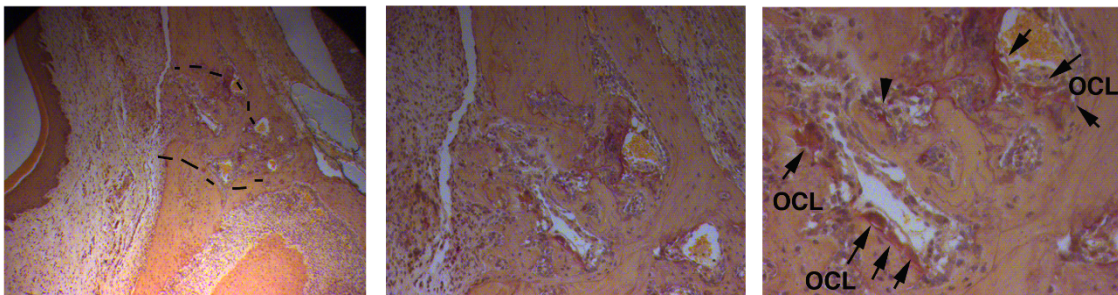


## Piezotome

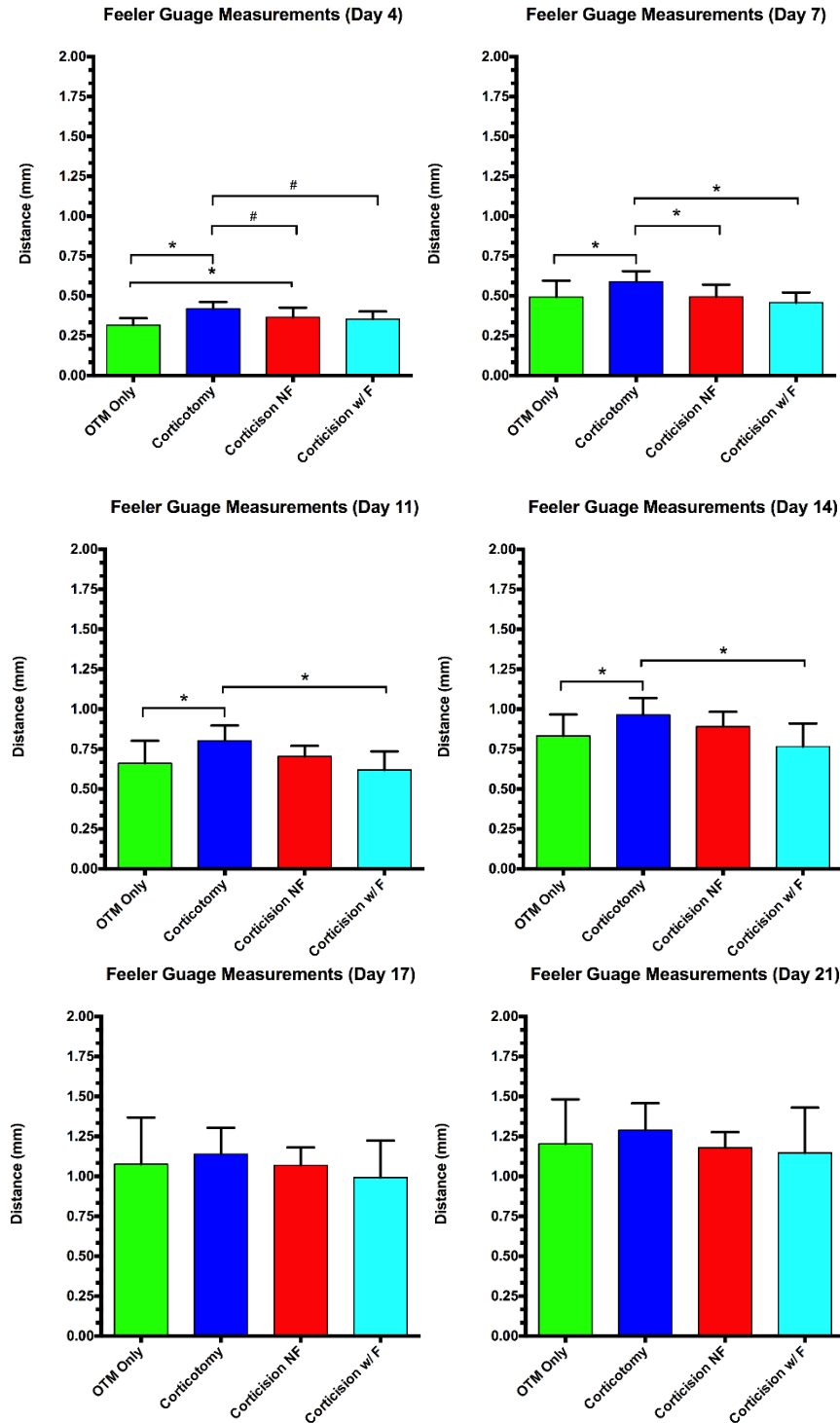
Day 4



Day 7

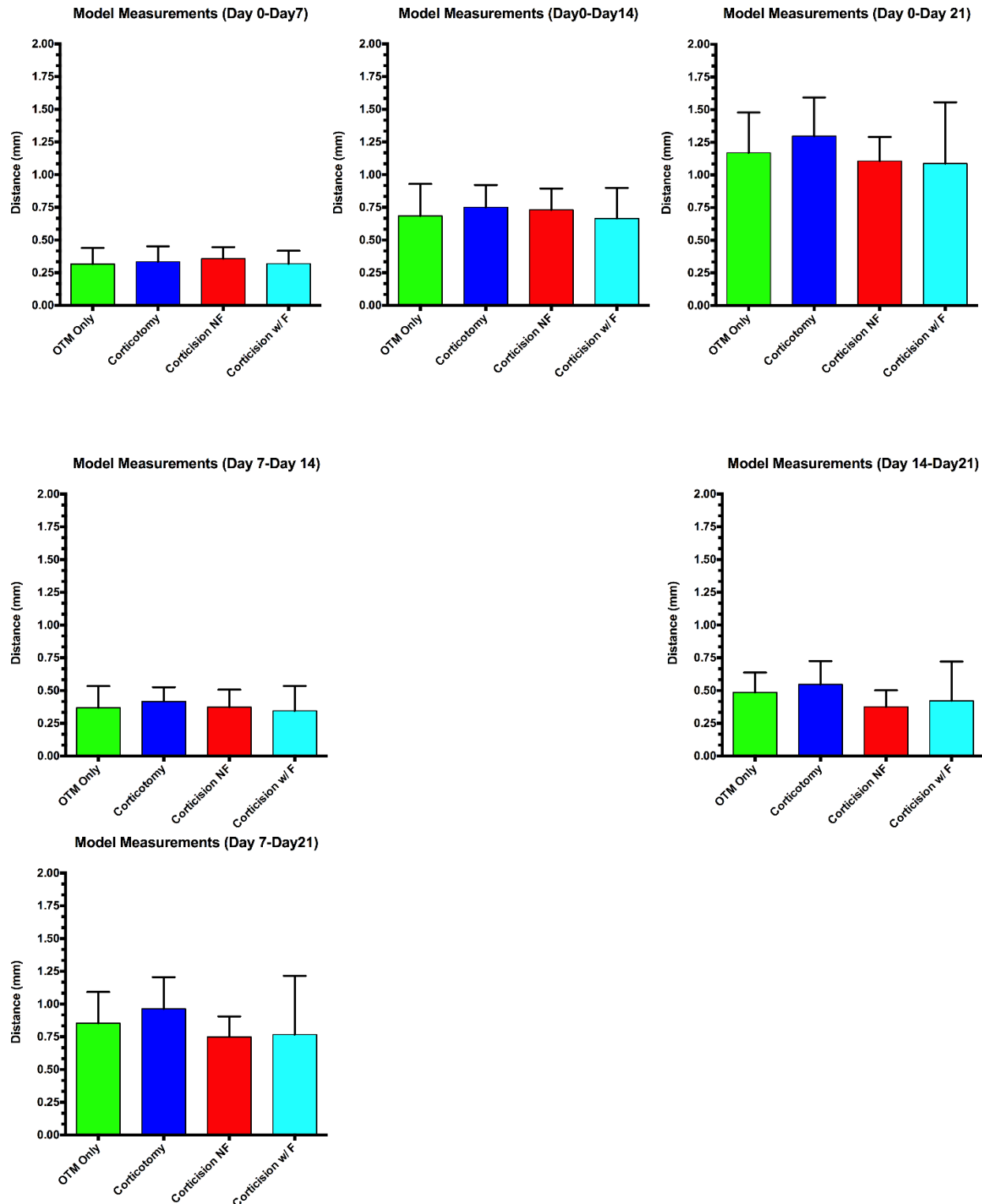


**Figure 13: TRAP staining.** TRAP staining to examine if there is osteoclast infiltration at the surgical site to show signs of the remodeling and resorptive process beginning and contributing towards the side effects seen, for example excessive tipping of the first molar. The piezocision group showed more osteoclasts at Day 7 than Day 4 and more osteoclasts than Day 4 in corticotomy group.



**Figure 14: Feeler Gauge Measurements Day 4, 7, 11, 14, 17, & 21.** During days 4, 7, 11, and 14 there were significant differences ( $p < 0.0001$ ,  $p = 0.0005$ ,  $p = 0.0004$ ,  $p = 0.0007$ ) between surgical groups and OTM only group but on day 17 and 21 there was no significant difference. This may indicate that the surgical procedures have an initial transient effect which eventually dissipates after two weeks has passed, further suggesting the need for more than one surgical procedure during treatment.



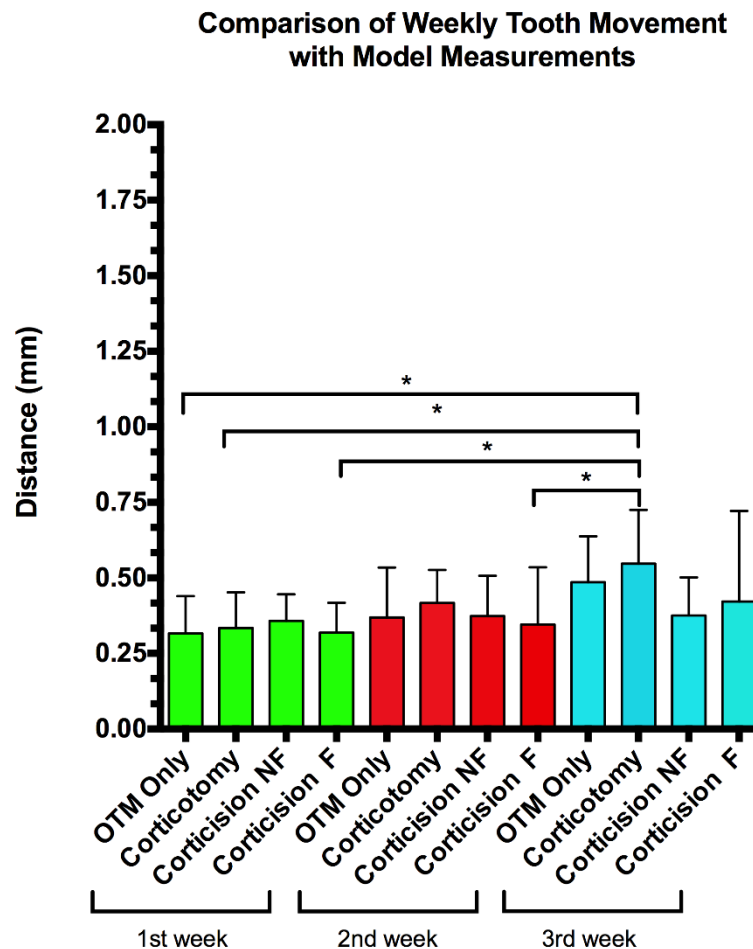


**Figure 15: Tooth Movement Measurements on Stone Models.** One, two and three week intervals were compared to identify if tooth movement was greater throughout a certain week. There was no significant difference in tooth movement between all groups at all time points. Day

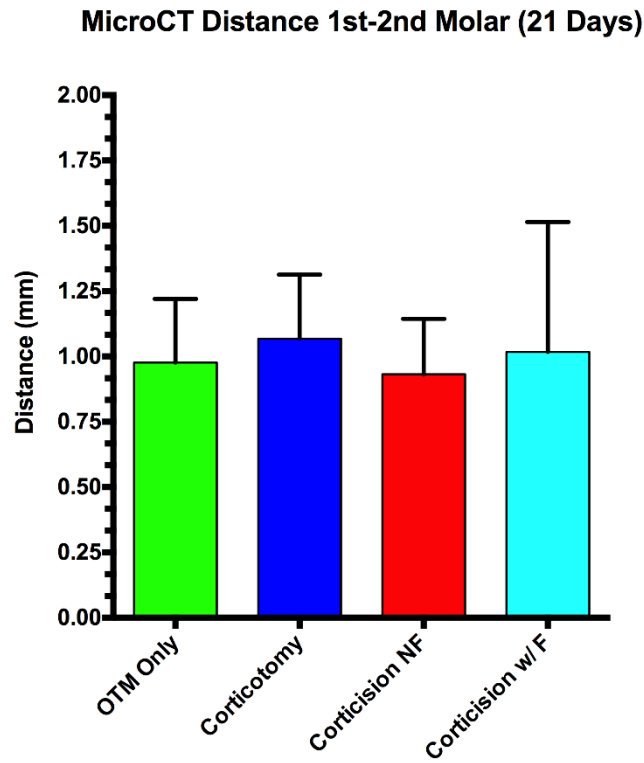
0-7 (p=0.737), Day 0-14 (p=0.675), Day 0-21 (p=0.3337), Day 7-14 (0.667), Day 14-21 (p=0.129), Day 7-21 (p=0.199).

Intraclass Correlation Coefficient							
	Intraclass Correlation <sup>b</sup>	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.990 <sup>a</sup>	.984	.994	205.371	63	63	.000
Average Measures	.995 <sup>c</sup>	.992	.997	205.371	63	63	.000

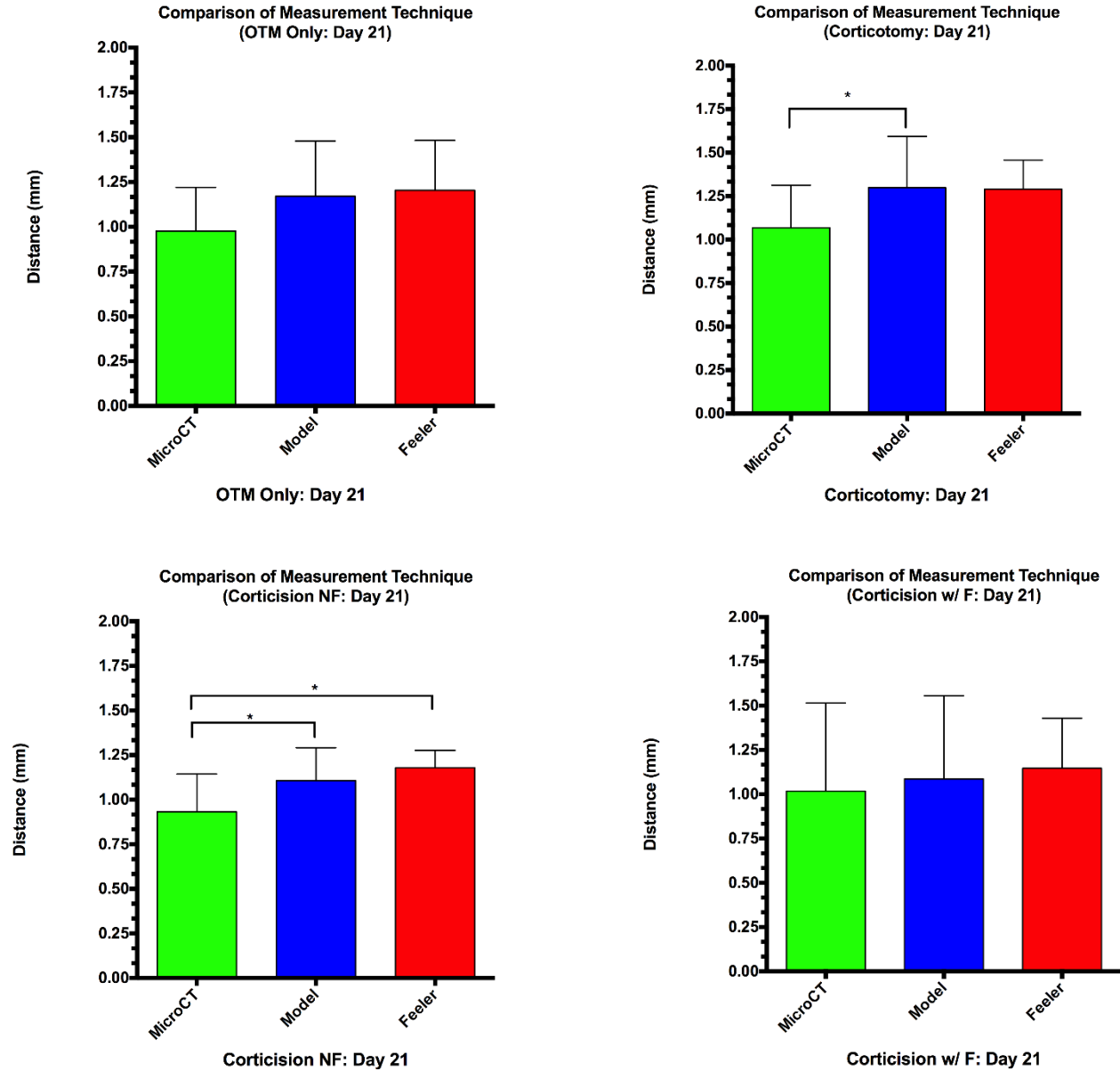
**Figure 16: Intraclass Correlation Coefficient.** Intra-rater reliability was tested and there was excellent reliability between measurements made on the stone models.



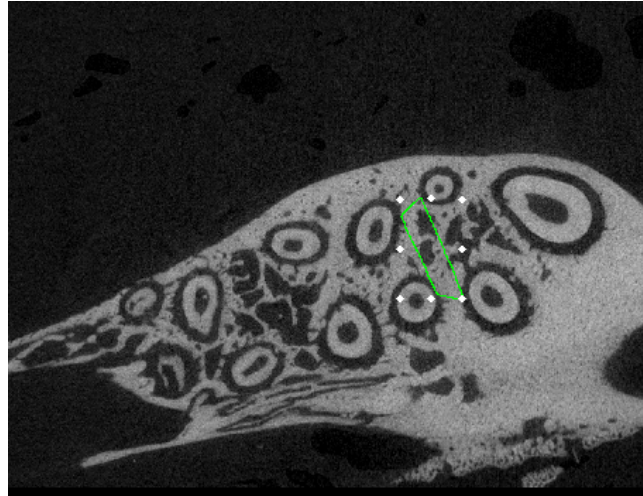
**Figure 17: Comparison of Weekly Tooth Movement with Stone Model Measurements.** The purpose of this was to identify if there was significant difference in tooth movement from week to week. There was a significant difference between groups (p=0.003). The third week of the Corticotomy group was significantly greater than the first week OTM only group, first week of the corticotomy group, and the first and second week of the Corticision with flap group.



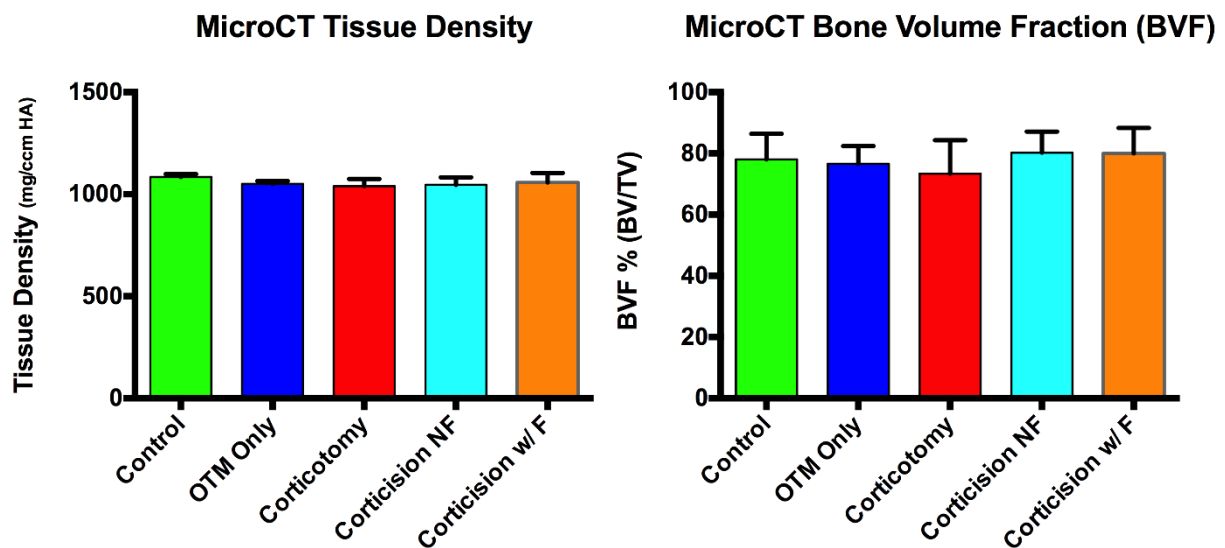
**Figure 18: MicroCT Tooth Movement Distance from 1<sup>st</sup> molar to 2<sup>nd</sup> molar.** There was no significant difference between all surgical modalities and OTM only ( $p=0.703$ ).



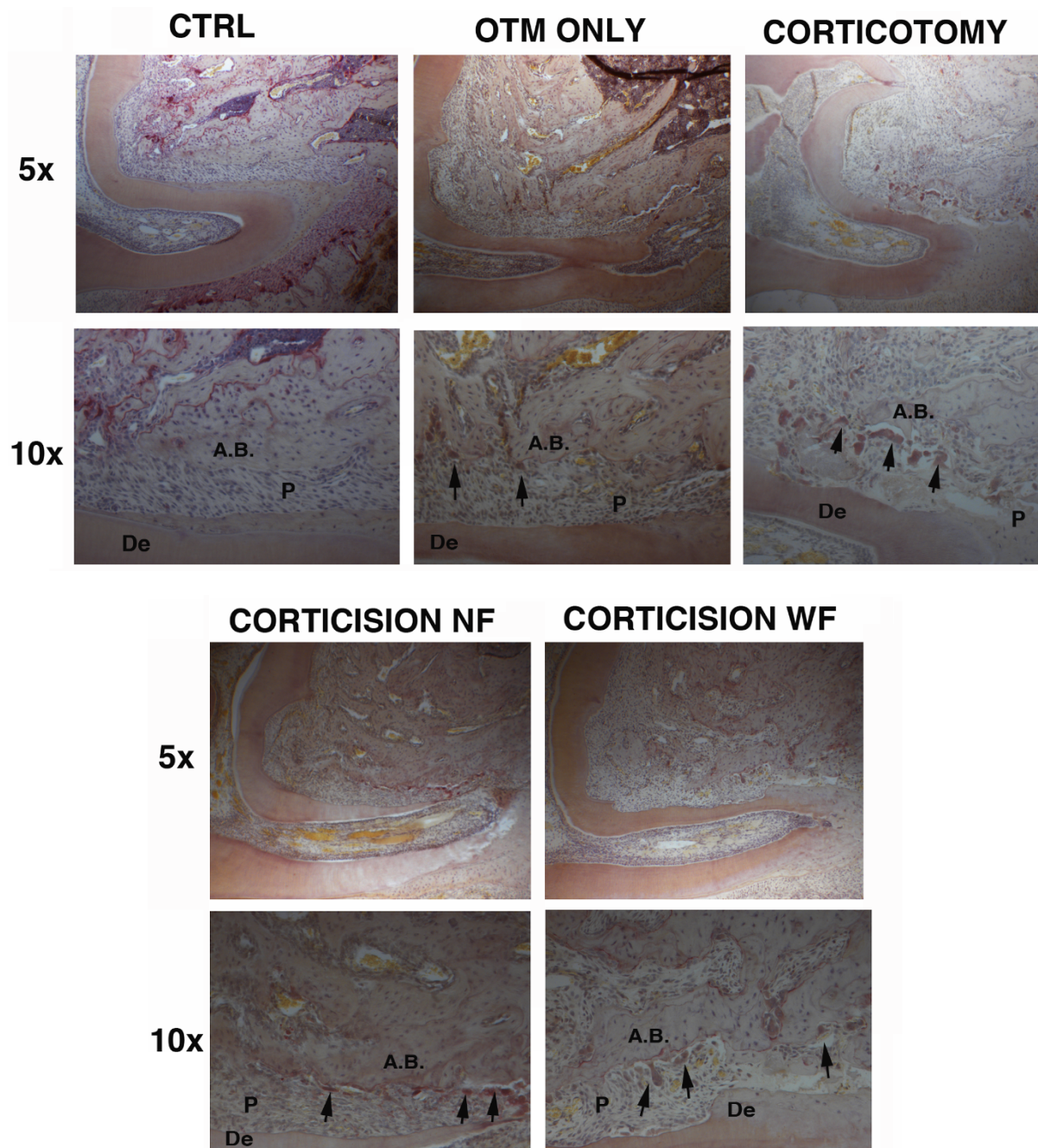
**Figure 19: Comparison of the Measurement Techniques to evaluate accuracy.** At day 21, there was a significant difference between measurement techniques in the Corticotomy group ( $p=0.0251$ ) and the Corticision no flap group ( $p=0.0016$ ). In the Corticotomy group microCT was significantly less than model measurements and in the Corticision no flap group microCT was significantly less than both Feeler gauge and stone models measurements. This shows that there is a possibility of inaccuracy between the groups.



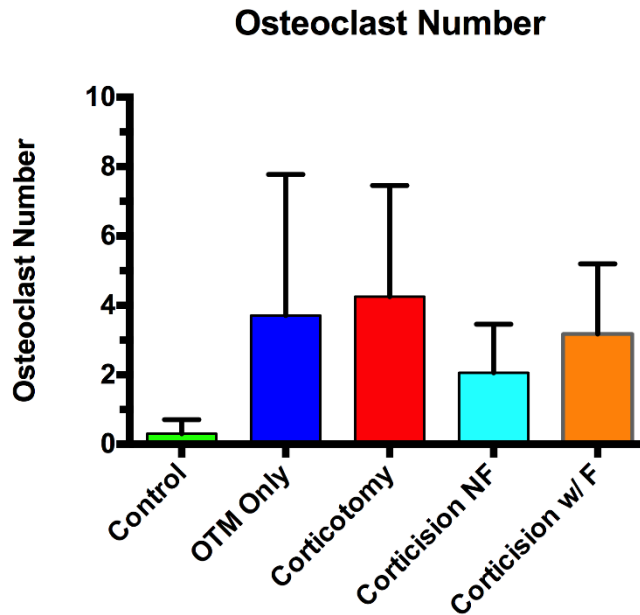
**Figure 20:** MicroCT image and the region of interest (ROI) for bone parameter measurements, Tissue Density and Bone Volume Fraction (BVF).



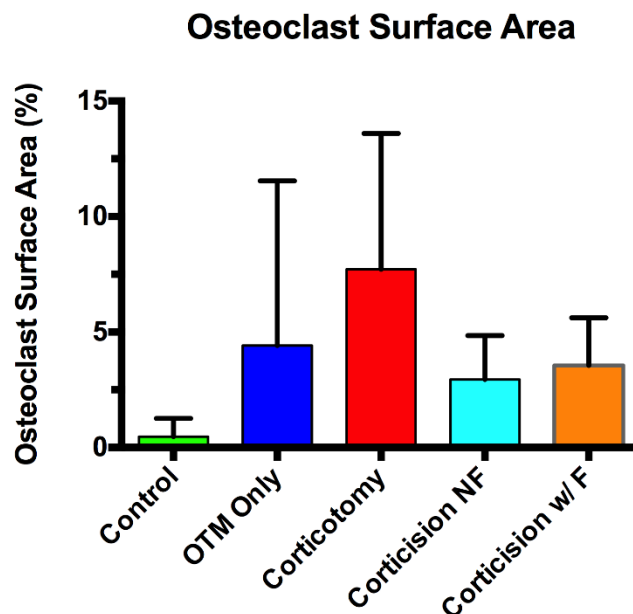
**Figure 21: MicroCT Tissue Density and Bone Volume Fraction.** At day 21, there was no significant difference between groups, including a control group with no tooth movement, for microCT tissue density ( $p=0.137$ ) and bone volume fraction ( $p=0.177$ ).



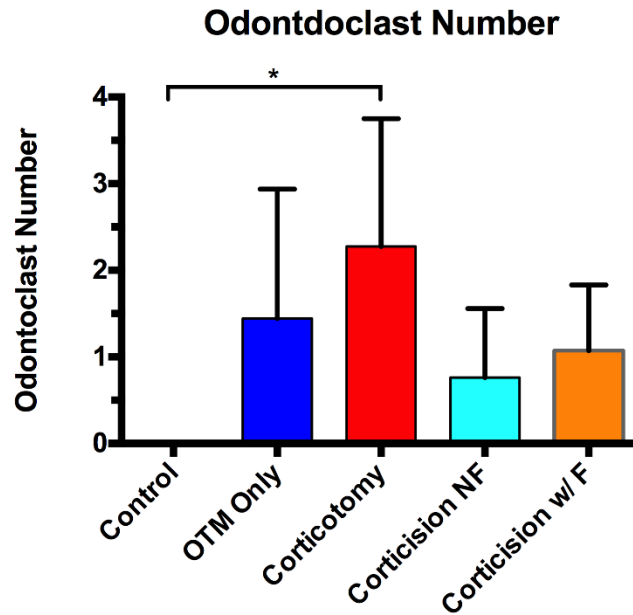
**Figure 22: TRAP stained sections.** Arrows point to TRAP positive cells lying on alveolar bone and dentin. A.B. = Alveolar bone. De = Dentin. P = PDL.



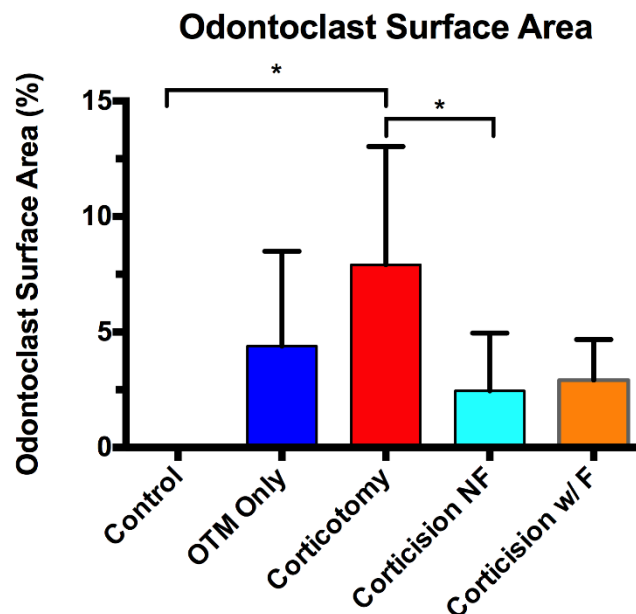
**Figure 23: Osteoclast Number.** Comparison of osteoclast number on day 21 measured using histomorphometry. ANOVA comparison of the groups demonstrated no significant difference ( $p=0.1321$ ) ( $n=7$ ).



**Figure 24: Osteoclast Surface Area:** Comparison of osteoclast surface area on day 21 measured using histomorphometry. Osteoclast surface area was determined as the surface of an active osteoclast touching the alveolar bone and then divided by total bone surface per defined area. ANOVA comparison of the groups demonstrated no significant difference ( $p=0.1062$ ) ( $n=7$ ).

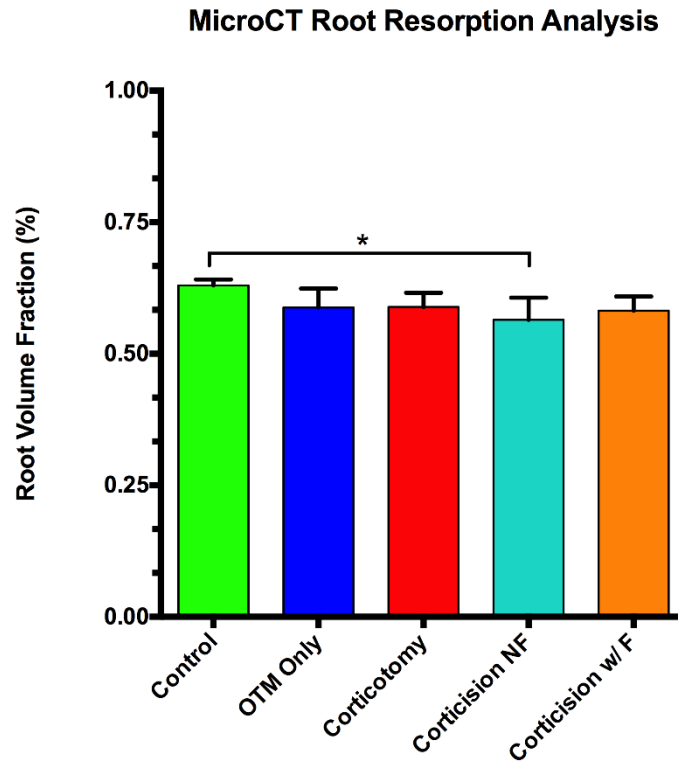


**Figure 25: Odontoclast Number.** Comparison of odontoclast number on day 21 measured using histomorphometry. ANOVA comparison of the groups demonstrated a significant difference between groups ( $p=0.182$ ) ( $n=7$ ). The Corticotomy group was significantly more than the control group.



**Figure 26: Odontoclast Surface Area:** Comparison of odontoclast surface area on day 21 measured using histomorphometry. Odontoclast surface area was considered as the surface of an active odontoclast touching the dentin and then divided by total dentin surface that extends from bifurcation to the end of the apex. ANOVA comparison of the groups demonstrated a significant difference between the groups ( $p=0.005$ ) ( $n=7$ ). The corticotomy group was significantly larger than the control and Corticision no flap groups.





**Figure 27: MicroCT Root Resorption Analysis.** There is a significant difference ( $p=0.0258$ ) between the groups. The Corticision with no flap group had a significantly less root volume fraction than the control (No force or OTM) group. This illustrates that the more invasive procedures might have a protective effect against root resorption.

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