THE EFFECTS OF CIRCUMFERENTIAL SUPRACRESTAL FIBEROTOMY ON

THE RATE OF TOOTH MOVEMENT IN THE BEAGLE MANDIBLE: A

RANDOMIZED SPLIT-MOUTH STUDY

A Thesis

by

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ABSTRACT

Introduction: The purpose of this study was to evaluate whether a single circumferential supracrestal fiberotomy procedure affects the bone around teeth and accelerates tooth movements. Methods: Seven beagle dogs were fitted with orthodontic appliances to protract the mandibular third premolars. Mandibular second premolars were extracted. Using a randomized split-mouth design, the experimental side was allocated to have a fiberotomy procedure around the mandibular third premolar. The same appliance and force systems were used on both sides. Tooth movements were analyzed over eight weeks using calipers and radiographs. The volume and density of bone mesial to the third premolars were analyzed using μCT. Bone remodeling was evaluated using histologic and fluorescent sections. Results: Tooth movements were not significantly different between the control and experimental sides. There also were no statistically significant differences in volume fraction or density of the medullary bone mesial to the third premolar. Histologic evaluations showed no consistent pattern of difference in osteoclast numbers between experimental and control sides, and the fluorescent evaluations showed similar patterns of bone modeling on both sides. Histologic evaluations of the soft tissues indicated that fibers were re-attached to the root surface eight weeks after fiberotomy, but they were smaller and less organized than control fibers. Conclusions: Circumferential supracrestal fiberotomy alone does not increase the rate of tooth movements because it has little effect on bone supporting teeth.

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NOMENCLATURE

μCT   Microcomputed Tomography

CSF   Circumferential Supracrestal Fiberotomy

RAP   Regional Acceleratory Phenomenon

PDL   Periodontal Ligament

NiTi   Nickel Titanium

MOP   Micro-Osteoperforations

H&E   Hematoxylin & Eosin

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The total treatment time for patients undergoing comprehensive orthodontics depends on a number of factors, including the distance teeth need to be moved and patient cooperation.[1,2] The American Association of Orthodontists states that orthodontic treatment can range from one to three years. On average, the treatment time for non-extraction cases is 24 months, and slightly longer, 28 months, for extraction cases.[3]

The risks involved with orthodontic treatment, including white spot lesion formation, root resorption, and decreased patient compliance, are increased with longer orthodontic treatment times.[4-8] It is in the best interest of the orthodontic professional and patient, therefore, to minimize time spent in treatment. A major consideration affecting the length of orthodontic treatment is the rate of tooth movement that can be achieved.

A variety of methods to increase tooth movement have been studied, including altering orthodontic mechanics or appliances, introducing adjunctive pharmacologic agents, and surgical interventions. Surgical methods, including corticotomies, generally work by induction of the regional acceleratory phenomenon (RAP) in the alveolar bone, but are invasive and costly. Attempts to find less invasive techniques that increase tooth movement include damage to soft tissue by raising a mucoperiosteal flap, and severing supracrestal fibers between the tooth and bone.

The following section will review the biology and rate of tooth movement, as well as discuss human and animal research regarding surgical and non-surgical methods to increase the rate of tooth movement. The regional acceleratory phenomenon will then be discussed. Next, corticotomies and other less invasive methods of inducing the RAP will be covered. Lastly, the literature regarding circumferential supracrestal fiberotomy in increasing the rate of tooth movement will be reviewed.

Biology of Tooth Movement

Tooth movement through bone takes place via bone modeling: resorption of bone occurs adjacent to the tooth in the direction of movement and deposition of bone occurs opposite the direction of movement.[9] There are several hypotheses that attempt to explain how this process takes place, including the piezoelectric hypothesis, the pressure-tension hypothesis and the mechanotransduction hypothesis. Though each of the hypotheses suggests separate mechanisms, it is probable that tooth movement actually occurs by a combination of these processes.

Pressure-Tension Hypothesis

Early research performed by Sandstedt[10] and Oppenheim[11] led to the development of the pressure-tension hypothesis. The hypothesis suggests that tooth movement occurs due to stretching and compression of the periodontal ligament (PDL). When force is applied to a tooth, the PDL undergoes tension on one side, the side opposite the direction of force, and compression on the other. Blood flow within the PDL increases due to tension and decreases due to compression. Under light force, the

osteoclasts in the bone adjacent to the compressed PDL can stimulate bone resorption, while bone deposition is stimulated in the bone adjacent to the stretched PDL. This process of bone resorption and formation allows the tooth to move through the bone in the direction of the applied force.

Piezoelectric Hypothesis

The piezoelectric hypothesis states that crystalline structures, such as bone, produce an electric potential in response to deformation from a force.[12] In 1962, Basset and Becker[13] found that the electric potential produced in bone after deformation can lead to bone formation. The electric potential causes a change in polarity at the surface of the bone with greater movement of positively charged ions to the pressure side and negatively charged ions to the tension side of bone during force application. The change in polarity of the bone, therefore, may by thought to play a role in signaling bone formation during normal function, but the signal dies away quickly, and the polarity reverses when the force is removed. Davidovitch et al[14,15] utilized the cat model to study tooth movement with and without application of constant electric current. They showed faster tooth movement with force and electric current, than with force alone. This hypothesis likely does not explain tooth movement under continued orthodontic fore because the change in electric polarity is not maintained after initial deformation.

Mechanotransduction Hypothesis

The mechanotransduction hypotheses, which probably best describes the mechanism of tooth movement, was discussed by Moss[16] in 1997. Central to the mechanism of mechanotransduction are bone cells known as osteocytes, which are

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located within bone spaces called lacunae. Osteocytes are interconnected through gap junctions that form an osseous canalicular system, and they have cellular processes that traverse the network to reach distant osteocytes and other bone cells. The hypothesis suggests that mechanical disturbances caused in bone by orthodontic force are converted into electric and/or biochemical signals that are transmitted through the canalicular system by the osteocytes. These signals reach osteoblasts and osteoclasts and can stimulate the process of bone modeling that leads to tooth movement.

Effect of Bone Remodeling and Bone Density on Tooth Movement

There is evidence of a relationship between the amount of tooth movement that occurs and the rate of bone remodeling. In 2000, Verna et al[17] studied tooth movement in Wistar rats divided into three groups: normal bone turnover, low bone turnover, and high bone turnover. Thyroid function was altered to induce hyperthyroidism in the high bone turnover group, and hypothyroidism in the low bone turnover group. Tooth movement was achieved via application of a constant orthodontic force on the maxillary first molar. After three weeks, the rates of total tooth movement were compared. Significantly faster tooth movements occurred in the high bone turnover group and slower tooth movements occurred in the low bone turnover group compared to controls.

There is also an association between bone density and tooth movement. Goldie et al[18], in 1984, studied the difference in tooth movement that occurred between control rats and calcium-deficient rats with low bone density. The amount of maxillary molar

movement was greater in the rats with lower bone density. They concluded that increased tooth movements were correlated directly with decreased bone density.

Ashcraft et al[19] also studied the effect of bone density on tooth movements in New Zealand white rabbits. Daily injections of corticosteroids were used to decrease bone density in the experimental group. Orthodontic force was applied in both groups over the course of 14 days, and the amount of tooth movement was measured. The results showed that animals with decreased bone density experienced three to four times the amount of tooth movement seen in controls.

Rate of Tooth Movement

A major determinant of orthodontic treatment time is the rate of tooth movement that can be achieved. A variety of studies, using both human and animal models, have been performed to measure the normal rates of tooth movement. The dog model has been especially useful for investigating different orthodontic forces and mechanics because dog and human bone and tooth structures are similar.[20,21] In addition, histological evaluation following tooth movement can be conducted when the dog model is utilized.

In the search for orthodontic mechanics that achieve optimum tooth movement, researchers have studied various force magnitudes,[22-25] as well as the effect of the continuity of force application[26-29] and of friction and binding between different bracket types.[30-32] The following sections will describe some of the clinical and animal studies

that have provided an understanding of the effects of orthodontic mechanics on tooth movement.

Force Magnitude

In a split-mouth clinical study, Boester and Johnston[22] compared rates of space closure during canine retraction using springs of 2, 5, 8, and 11 ounces of force (approximately 55, 140, 225, and 310 grams of force). Springs were in place for ten weeks and were reactivated each week. The results showed that space closure was significantly slower in the 2-ounce force group. However, there were no significant differences in the rates of space closure among the remaining groups: forces from 5-11 ounces. The authors proposed that within this force range, the rate of bone resorption could be at a maximum level and thus be the rate-limiting factor.

Iwasaki et al[23] conducted a split-mouth human study to analyze the effect of force levels. Maxillary first premolar extraction spaces were closed using NiTi closing coils with continuous forces of 18 grams and 60 grams. Nance appliances and co-ligation of the first molars with the second premolars were used as anchorage to allow tooth movement the canine to be studied alone. The findings showed 1.3 mm of tooth movement per month for the 60 g group and 0.9 mm per month for the 18 g group. These differences in space closure rate were statistically significant. It was noted that the major differences in tooth velocities occurred during the initial three months, with no significant differences thereafter.

Using a split-mouth design in the dog model, Pilon et al[25] studied distalization of mandibular second premolars using forces of 50, 100, and 200 g applied from pre-

stretched elastic modules. The authors discriminated four phases of tooth movement from the resulting time-displacement curves: the initial tooth movement phase, the arrest of tooth movement phase, the acceleration of tooth movement phase, and the constant linear tooth movement phase. No significant difference in tooth movement rates were found between the three force levels during any of the phases, nor were there differences in the lengths of the phases between groups. Differences in tooth movement were identified between the different animals, but control and experimental sides on each individual animal were highly correlated. Thus, tooth movement rate was independent of force magnitude at the levels tested (50, 100, and 200 g).

Owens et al[24] evaluated retraction of mandibular second premolars after extraction of third premolars in dogs. Miniscrew implants were placed adjacent to the fourth premolar and forces were applied directly from the miniscrews to the second premolar using NiTi coils with forces of 25 and 50 grams. No significant differences in tooth movements were detected. Experimental and control sides showed an average rate of 0.25 mm of retraction per week.

Continuity of Force

In a clinical study, Samuels et al[29] evaluated how the continuity of force application affected rates of space closure. NiTi closing coils were used to achieve a light continuous force of 150 grams, while elastomeric chains were used to achieve a heavier intermittent force of 400 to 450 grams. The continuous force closed space at 0.26 mm per week, while the intermittent force closed space at 0.19 mm per week. In a follow-up study, Samuels et al[27] demonstrated that 100 g and 200 gram of continuous

force closed space at 0.16 mm and 0.24 mm per week, respectively. Combining the results of these studies, the 100 g continuous force and the 400 to 450 g intermittent force produced significantly lower rates of tooth movement than the 150 and 200 g continuous forces, which were not significantly different from each other.

In another clinical study, intermittent versus continuous space closure was compared by Daskalogiannakis and McLachlan.[26] Space closure was performed on one side with a 70 g vertical retraction loop that was activated every six weeks (intermittent). On the opposite side, space closure was performed using the same loop and the addition of 60 g rare earth magnets (continuous). The intermittent force closed space at 0.63 mm per month while the continuous force closed space significantly faster, at 1.22 mm per month.

Van Leeuwen et al[28] used beagles to evaluate the difference between light continuous and light intermittent forces. Mandibular third premolars were extracted and second premolars were retracted into the space using NiTi springs that produced 10.2 and 25.5 g. The springs were either left in continuously or for 16 hours per day for the duration of the 120-day experiment. The authors found that tooth movements exhibited four phases, similar to those mentioned previously: initiation, arrest, acceleration, and linear. During the linear phase, the continuous force groups of 25.5 g and 10.2 g exhibited space closures of 0.37 mm and 0.32 mm per week, respectively. For the same force levels, the discontinuous mechanics closed space at 0.25 and 0.21 mm per week, respectively. These findings suggest equivalent continuous forces closed space faster than intermittent forces during the linear phase of tooth movement.

Friction and Binding

A common method of space closure involves moving a tooth or group of teeth along the arch wire as the wire slides through the bracket slots. This type of space closure can be influenced by friction and binding between brackets and archwires. In an attempt to assess the role of friction and binding, Burrow[30] conducted a split-mouth study comparing self-ligating brackets (Damon3 and SmartClip) and conventional twin brackets (Victory Series). Victory Series brackets were placed on all teeth except for the experimental canine, and a transpalatal arch was used for stabilization. Canines were retracted on a 0.018-inch stainless steel archwire with 150 g NiTi retraction springs. Over the four-week study, tooth movement with the conventional bracket was 1.2 mm. Rates for self-ligating brackets were 0.9 mm (Damon3) and 1.1 mm (SmartClip) over the four weeks. The differences between the two bracket types were statistically significant. In the conclusions, the author postulated that although self-ligating brackets might be expected to introduce less friction and have a faster rate of tooth movement, the geometry of the brackets might have played a role. The self-ligating brackets were narrow, which could have increased binding with the archwires.

Other studies evaluating the relationship between ligation and rate of tooth movement have found no differences. Scott et al[32] showed no difference in efficiency of initial tooth alignment in a randomized controlled trial between patients with self-ligating brackets versus conventional brackets. Similarly, da Costa Monini et al[31] found no difference in the rate of canine retraction between conventional and self-ligating appliances in a split-mouth investigation.

Summary

In 2003, Ren et al,[33] performed a systematic review of the literature pertaining to orthodontic tooth movement in animals and humans. The goal of the review was to evaluate the evidence relating rates of tooth movement to the forces used. The authors found that large differences in tooth movement can occur between subjects and within individuals, even when consistent mechanics and force levels are used. Additionally, even with significantly different forces, tooth movement can be equivalent between and within individuals. Ren et al[33] hypothesized that these seemingly ambiguous results are possibly a consequence of individual variation in a number of contributing factors, such as cytokines and growth factors, or in anatomy and cellular response to tooth movement.

In a subsequent systematic review, Ren et al[34] combined numerical data to develop a mathematical relationship between tooth movement and force in both beagle dogs and humans. Their dog model predicts second mandibular premolar movement of 0.27 mm per week with an optimum force of 253 g. The exact force value was not critical; the 95% confidence interval ranged from 106 to 463 g. Their human model predicted that canines retract 0.29 mm per week under 277 g of force, with a 95% confidence interval of 135 to 471 g. Ren et al[34] concluded that human and dog tooth movement rates are not significantly different and that they can be achieved over a wide range of forces, which also are not significantly different.

The findings of Ren et al,[33,34] in combination with results from other previously discussed studies on tooth movement, indicate that teeth move at a rate of approximately 1 mm/month with some variation expected within and between individuals.[26,27,30] Thus,

for an orthodontic patient with a 7 mm space following premolar extraction, space closure may be predicted to take approximately 7 months.

Adjunctive Therapies to Increase Tooth Movement

Several investigators have attempted to increase the rate of tooth movement by administering adjunctive therapies that speed up the biologic processes responsible for tooth movement. These therapies often involve activation of bone resorbing cells, osteoclasts, and include administration of vitamin D, osteocalcin, or prostaglandin.[35-37] As previously mentioned, the rate of tooth movement has been linked to the rate of bony remodeling. More specifically, increased tooth movement occurs when bone density is lower and bone turnover is high. In light of this, researchers have looked at different means of increasing the rate of bone resorption to allow for faster tooth movement.

Collins and Sinclair[35] studied the effect of localized injections of 1,25-dihydroxycholecalciferol (active vitamin D), a stimulator of osteoclastic activity, on the rate of tooth movement in cats. Using a split-mouth design, each cat received an injection of vitamin D, dissolved in dimethylsulfoxide, into the PDL on the experimental side. Dimethylsulfoxide alone was injected into the PDL on the control side. All injections were given at the distal aspect of the canines and the canines were retracted orthodontically using an 80 g force. After 21 days, the experimental teeth had moved 60% further than the control teeth. The histologic evaluations showed an increase in the number of osteoclasts on the experimental side, consistent with the role of vitamin D as a stimulator of osteoclast activity.

Kobayashi et al[36] studied the effect of local administration of osteocalcin, a bone matrix protein involved in the recruitment and differentiation of osteoclasts, on the rate of tooth movement in rats. They found a significant increase in the rate of tooth movement and number of osteoclasts present after local administration of osteocalcin. Yamasaki et al[37] performed a clinical study evaluating the effect of local administration of prostaglandin E1, a biochemical mediator of bone resorption in both monkeys and humans. Their results also showed an increase in the rate of tooth movement following prostaglandin E1 injection compared to controls.

Although each of the three above mentioned studies found an increase in the rates of tooth movement, they all performed multiple injections, over a relatively short period of time (ranging from 3-26 days). Weekly appointments would be costly and time-consuming, and may not be realistic for a patient or their orthodontist. In addition, the systemic and long-term effects of vitamin D, osteocalcin and prostaglandin E1 injections were not evaluated.

Regional Acceleratory Phenomenon

Tooth movements can also be accelerated by the regional acceleratory phenomenon (RAP). The RAP, first described by Frost, involves inflammation following injury to bone.[38] The inflammatory reaction leads to a cascade of signaling molecules that eventually causes osteoclastogenesis, decreased bone density and increased bone turnover near the injury.[38-40] The RAP is a reaction of tissue to noxious stimuli, which could include a crushing injury, a fracture, or an operation. If increased tooth movement

is desired, bone injury can be performed near a tooth of interest and the decrease in bone density in the area allows for faster bone resorption and faster tooth movement. Tooth movements have been shown to increase by two to five times after producing the RAP with corticotomies.[41-43] These effects last for approximately 1-2 months in dogs and 2-3 months in humans.[3]

The extent of the RAP is related to the amount of tissue insult. McBride et al[44] demonstrated a greater decrease in bone density on the side with more extensive bone injury than on the side with less damage. Cohen et al[45] showed that increasing the extent of bone damage increases the rate of tooth movement. Many researchers have studied different methods of inducing the RAP to achieve faster tooth movement; one of the most common procedures currently used is corticotomies.

Corticotomies

Corticotomies, surgical cuts made into the cortex of the alveolar bone, have been used to enhance the rate of orthodontic tooth movement since the late 1950's.[46-48] The procedure was popularized by the Wilcko brothers in 2001. The Wilckos emphasized that corticotomies increase the rate of tooth movement by inducing the RAP.[49] In their original protocol, orthodontic appliances are placed, full-thickness mucoperiosteal flaps are elevated from a coronal approach, and vertical corticotomies are made between the teeth extending from 2-3 mm apical of the alveolar crest to 2 mm beyond the tooth apices and connected by a scalloped subapical corticotomy. This was performed on both the labial and lingual aspects of the alveolar bone. After all cuts had been completed, a

bone allograft was applied and the soft tissues were re-approximated. Using this protocol, they have reported cases with treatment times as short as six months. Although the Wilcko brothers report faster tooth movement, their conclusions are drawn from case studies without controls.[49,50]

In a split mouth study using beagle dogs, Cho et al[41] compared tooth movement with or without corticotomies, following 4 weeks of post-extraction healing. They found that corticotomies plus orthodontic force moved teeth two times faster in the mandible and four times faster in the maxilla than orthodontic forces alone. In addition, the authors noted that maximum tooth movement occurred two weeks post-corticotomy.

After allowing 16 weeks for extractions sites to heal, Iino et al[42] also found faster tooth movement with corticotomies in dogs. Tooth movements were twice as fast during the first week and five times as fast during the second week. The overall result at the end of four weeks was twice the amount of tooth movement.

Sanjideh et al[43] found that corticotomies performed the same day as extractions also produced twice as much tooth movement. They also found that a second corticotomy performed 4 weeks later produces statistically significant differences in total tooth movement, but the difference was not clinically significant, suggesting that a second corticotomy procedure is not warranted.

The RAP produced by corticotomies is a localized phenomenon, with no cross-over effect to the other side of the arch. A rat study showed that selective alveolar decortication induced increased turnover of alveolar spongiosa that is localized to the area immediately adjacent to the decortication injury.[51]

Flapless Bone Damage

The corticotomy procedure incurs some risks to patients, including potential loss of alveolar bone height[52] and possible dehiscence in areas of thin alveolar bone. Due to these risks and the expense of surgery, investigators have sought alternate methods of achieving the RAP through less invasive means. Several of these techniques attempt to achieve bone injury through the mucosa, without raising a flap. Flapless approaches have induced bone injuries using a variety of instruments, including a surgical bur,[53] a piezoknife,[54-58] a scalpel,[59] or a bone awl.[60] More recently, micro-osteoperforations (MOPs) have been created with the PROPEL device.[61,62]

A dog split-mouth study by Safavi et al[53] investigated flapless corticotomies induced with a surgical bur. The corticotomies were produced by drilling 2 mm deep holes with a fine surgical fissure bur through the buccal cortex mesial, buccal and distal to the second premolar. The second premolars were immediately loaded for mesialization into first premolar extraction spaces using a 150 gram NiTi spring for 3 months. Corticotomies were performed immediately after extraction, at 1 month, and at 2 months. Results showed that the experimental premolars moved 0.82 mm more than the control premolar over the first month. However, there were not significant differences in tooth movement during the second month. In the third month, the premolars on the experimental side moved 1.15 mm less than the control side. Over the three month study period, there was no significant difference in the amount of tooth movement between groups.

Swapp et al[60] conducted a dog-study in which 60 microfracture injuries were induced in the buccal and lingual cortical plates around a mandibular tooth with a bone awl. After 4 weeks of extraction site healing, microfractures were created around the experimental tooth roots and extending 5 to 6 mm into the extraction space. Target teeth were then moved orthodontically. Although microcomputed tomography (μCT) results showed significant decreases in cortical bone volume fraction and cortical bone density on the experimental side, medullary bone showed no difference in bone volume fraction or density. Histomorphometric images demonstrated cortical bone remodeling on the experimental side, but not on the control side. They found no difference in the rate of tooth movement between the experimental and control sides.

Cramer et al[62] studied the effect of micro-osteoperforations, made using the PROPEL device, on the rate of tooth movement in beagles. Maxillary second premolars were retracted into the third premolar extraction sites after 1 month of healing. The experimental side of the maxilla received 8 MOPs around the second premolar without a flap, while the opposite side of the maxilla received no surgery. Orthodontic force was applied to the second premolars using a 200 gram NiTi spring for 7 weeks. Intraoral and radiographic measurements of tooth movement over the course of the study were not statistically different. No significant differences were found in bone density or bone volume of the medullary bone after the 7 week course of tooth movement, indicating the flapless process of creating damage to the cortical bone did not produce a significant RAP effect.

Periosteal Flaps and the RAP

When studying the RAP, it is important to understand the effect that raising a mucoperiosteal flap has on the bone. According to Frost,[38-40] the effects of the RAP reflect regional differences in vascular anatomy and innervation. Regional vascularization of the alveolar bone comes from the medullary bone and the overlying periosteum. When the periosteum is stripped away during flap procedure, a large portion of the blood supply to the bone is disrupted. This disruption may, in part, be responsible for bony changes seen during corticotomy procedures.

In a 1994 study by Yaffe et al,[63] the RAP was shown following elevation of a full-thickness mucoperiosteal flap in rat mandibles. The 60 Wistar rats used in the study were divided into three groups: a control group, a full-thickness mucoperiosteal buccal flap group, and a full-thickness mucoperiosteal buccal and lingual flap group. Rats were sacrificed 7, 10, 14, 17, 21, and 120 days after surgery. The authors noted resorption of the alveolar and basal bone, with more extensive resorption in rats that had both buccal and lingual flaps elevated. Maximum resorption was seen three weeks after surgery and bone volume appeared to return to almost control levels 120 days after surgery. These findings are encouraging because a flap procedure is much less invasive than decortication and, yet, may produce the bony changes needed for increased tooth movement.

Owen et al[64] evaluated the effect of mucoperiosteal flap elevation on bone characteristics and the rate of tooth movement in beagles. Mandibular second premolars were extracted, and orthodontic appliances were placed for protraction of the third

premolars. The experimental side of the mandible had a full-thickness mucoperiosteal flap elevated on the buccal aspect of the alveolar bone, extending from the distal of the third premolar to the mesial of the first premolar. The control side received no surgery except extraction. Third premolars were protracted over 8 weeks. Intraoral caliper and radiograph measurements of tooth movement indicated that the experimental teeth moved 25% and 31% further than control teeth, respectively. Analysis of medullary bone density mesial to third premolars showed an 8% decrease in bone density on the experimental side. These differences were significant, and indicate that flap elevation alone can induce faster tooth movement.

In order to understand what aspect of the flap procedure might cause faster tooth movement, it is helpful to evaluate some of the periodontal literature. During a periodontal surgery, it is desired to maintain and sometimes increase the amount of bone supporting the dentition and minimize the amount of bone loss after surgery. Several different flap designs have been evaluated to identify bony changes following flap procedure.

Significance of the Marginal Gingiva

Studies investigating the effects of gingival flap surgery indicate that alveolar bone resorption occurs to a greater extent when the flap is raised from the coronal aspect than from the apical aspect.[65] In the coronal approach, marginal gingiva (the most coronal gingiva, attached to cementum) is removed from the tooth surface, whereas, in the apical approach, the marginal gingiva is left intact. These results suggest that the

marginal gingiva plays an important role in the process of bone resorption following gingival trauma.

Binderman et al[65] divided 18 Wistar rats into two groups: one group had mucoperiosteal flap surgery using a coronal approach, while the second group had surgery using an apical approach. Each group had buccal and lingual flaps performed on the experimental side of the mandible. The other side of the mandible served as the untreated control. Tissues healed for 21 days prior to animal sacrifice. Results showed extensive resorption of the periodontal aspect of the alveolar bone in the coronal approach group. Using histologic analysis, the investigators found 46% of the coronal approach group showed mild to severe vertical alveolar bone loss. In the apical flap group there was only slight resorption of the alveolar bone at the level of the mucosal incision, with some modeling of new trabecular bone coronal to the incision. Approximately 13% of the sections from the apical approach group showed mild vertical alveolar bone loss- significantly less than the bone loss seen in the coronal approach group. The authors suggested that the increased vertical alveolar bone loss in the coronal flap group might have been due to the injury of the marginal gingival connective tissue.

In 2002 Binderman et al[66] published their hypothesis regarding the relationship between the marginal periodontal tissues and bone resorption. They suggested that loss of connective tissue attachment between tooth roots and gingiva and between tooth roots and alveolar bone, like that which occurs in periodontitis, causes a strain-relaxation of the fibroblast cells that comprise the collagen fibers of the initial attachment. This strain relaxation produces a site-specific signal for alveolar bone resorption by initiating an

inflammatory cascade of signaling molecules. The authors indicated that the bone

resorption seen in patients with periodontitis may occur by the same mechanism as the

bone resorption seen in their 2001 study. Further research by Binderman et al[67]

regarding the signaling cascade, found significant up-regulation (2.8-fold) of the

purinoreceptor P2X4 gene following coronal surgical approach compared to apical

approach. P2X receptors have been linked to the generation of osteoclasts via up-

regulation of osteoblast-expressed receptor, an activator of RANKL,[68] and is indicated in

the formation and activation of osteoclasts.[69]

Nishio et al[70] studied the expression of certain proteins during the formation and

regeneration of the junctional epithelium (JE), the epithelium which lies at the base of

the gingival sulcus. They showed that proteins, odontogenic ameloblast-associated

(ODAM) and amelotin (AMTN), were expressed in the JE only during its initial

formation and during regeneration following damage to the gingival tissues by

gingivectomy. ODAM and AMTN proteins are encoded by two genes that are members

of the secretory calcium-binding phosphoprotein (SCPP) gene cluster, which encode for

various proteins related to the stabilization of calcium and phosphate ions in body fluids

and/or calcium phosphate deposition onto receptive extracellular matrices.[71] It is

possible that damage to the gingival tissue and JE that occur during flap elevation from a

coronal approach would stimulate expression of proteins that play a role in tissue

calcification.

These studies indicate that the bone resorption and faster tooth movements observed with flap surgery alone could have been due to damage to the marginal gingiva rather than disruption of blood supply from the periosteum.

Circumferential Supracrestal Fiberotomy

If damage to the marginal gingiva produced sufficient amounts of bone resorption to increase tooth movement, it would significantly reduce the amount of trauma necessary to achieve shorter orthodontic treatment times for patients. One procedure, circumferential fiberotomy, has been suggested as an alternative method of increasing the rate of tooth movement. The CSF procedure can be performed in the orthodontic office, with few negative side effects for the patient.[72,73]

Fiberotomy has been used in orthodontics to reduce relapse following orthodontic correction of tooth irregularities, namely rotations.[72] The procedure was introduced by Edwards, and was later studied in detail and coined circumferential supracrestal fiberotomy (CSF) by Campbell.[74] A surgical blade is inserted into the gingival sulcus to the crest of the alveolar bone surrounding a tooth in order to sever the fibers that attach the tooth surface to the surrounding soft tissue and adjacent teeth. Fibers attached from tooth to gingiva become stretched when a tooth is de-rotated with orthodontic appliances.[75] Studies evaluating the CSF procedure indicate that severing the supracrestal fibers around a rotated tooth tends to release the "pull" exerted by the attached tissue, thus relieving the force assumed to lead to relapse.[72] Fiberotomies can be and are performed in the orthodontic office, with the use of local anesthetic. Post-

procedure suturing and/or surgical dressing are not usually indicated, and post-op pain is manageable with over-the-counter analgesics.

Several animal studies have been performed to analyze the effect that fiberotomies have on the rate of tooth movement. In 1983, Glenn et al[76] used a feline model to compare the rate of canine retraction after fiberotomy to no surgery. The study lasted for 6 weeks and utilized orthodontic elastics (approximately 57 g of force) to tip canines distally into an edentulous area. Each week, fiberotomies were performed, a wedge of gingival tissue distal to the retracting tooth was removed and new elastics were placed. Measurements were made at the cusp tip of the canines. Results showed a significant difference between the groups, experimental teeth moved 108% more than control teeth. This study involved more gingival damage due to removal of ridge mucosa and weekly fiberotomy procedures, and it is difficult to extrapolate the results that might occur in a larger animal and with less gingival damage.

Tuncay et al,[77] in 1986, utilized a rat model to test for differences in tooth movement between a fiberotomy group and a control. Retraction force of 25 g was applied between the rat molar and incisor using nickel titanium closing coils. The study continued for 30 days, with fiberotomy being performed every third day (ten times total). In addition to circumferential fiberotomy, surgery involved an extension of the incision onto the alveolar ridge in the direction of tooth movement. This study found significantly increased rate of tooth movement in the group receiving fiberotomy (23% faster than control). Due to the frequency of fiberotomy and extension of incision onto the ridge, this study has the same limitations as the previous animal study. In addition,

the 25 grams of force used to retract the rat incisor in this study is far beyond the scale of a typical orthodontic force on a human tooth. The results of this study are not suitable for comparison to larger mammals.

In 2013, Young et al[78] used the rat model to show that fiberotomies produce higher rates of tooth movement and greater alveolar bone resorption than no surgery and apical flap procedures. The 34 rats used were divided into three groups: 1) fiberotomy alone (13 rats), 2) apical mucoperiosteal flap (marginal gingiva left intact – 14 rats), and 3) control (no surgery – 7 rats). All surgical procedures were performed bilaterally on the first molars, and the left side served as a control with no orthodontic force applied. Following surgery, a buccally directed force was applied to the molars for 14 days using a 0.012 NiTi wire (the magnitude of the force was not reported). The force was then removed and relapse was allowed to occur for an additional 16 days. Tooth movement measurements were made intraorally between the central grooves of the right and left first molars prior to surgery and then again at days 7, 14, 21 and 30. Results showed that the fiberotomy group exhibited the fastest rate of tooth movement (approximately twice that of the other two groups), the greatest amount of bone resorption (measured at the PDL surface of the buccal cortical plate) and the least amount of relapse (12% compared to almost 100%) of the three groups. It is important to note that the apical flap surgery, in which the marginal gingiva is left intact, did not increase the rate of tooth movement to the extent achieved by fiberotomy alone. The buccal tooth movement in this study is likely primarily tooth tipping and the results may not accurately estimate the most

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desirable type of orthodontic tooth movement (bodily movement) which usually occurs in a mesio-distal direction along the alveolar ridge.

Kalra et al[79] performed CSF in a split-mouth clinical study of 14 patients who underwent canine retraction following extraction of first premolars. One day after extraction of either maxillary (9 patients) or mandibular (5 patients) first premolars, orthodontic appliances were placed and retraction of canines was initiated using composite T-loop wires activated to produce 200 g of force from the first molars to the canines. Anchorage of the first molars was achieved by Nance appliances in the maxillary arches and lower lingual arch appliances in the mandibular arches. Impressions of the arches were made prior to appliance placement and at records dates 30 days, 60 days and 90 days following initiation of canine retraction. The T-loop was re-activated at each of the records dates. Total tooth movement was measured by superimposing a digitized version of the models made from these impressions. Measurements were made from the center of the occlusal surface of the first molar to the cusp tip of the canines. On average, results in the maxillary arch indicated that the fiberotomy side closed 2.50 mm, while the non-fiberotomy side closed 2.14 mm. Results in the mandibular arch indicated that the fiberotomy side closed 2.04 mm, while the non-fiberotomy side closed 1.44 mm. The differences in tooth movement between sides were not statistically significant in the maxilla or mandible. Problems with this study include the use of mechanics that are difficult to standardize as well as measurements of tooth movement that do not differentiate between bodily tooth movement (translation) and tipping.

The animal CSF studies have utilized small animal models that are difficult to

relate to humans due to high forces levels and greater amounts of tissue damage relative

to body size, as well as differences in bone and tooth structure. In addition, many studies

perform surgical damage in conjunction with fiberotomy, or perform fiberotomy

multiple times over a short period of time, which make their results less applicable to a

clinical scenario. The only clinical CSF study available did not provide adequate

information on standardization of mechanics and had a relatively small sample size with

no indications of population variability. It is, therefore, necessary to observe the effect of

a single fiberotomy on tooth movement in a well-controlled, large animal model that

more closely matches human bone and tooth structure and tooth movement mechanisms.

CHAPTER II

MATERIALS AND METHODS

Seven skeletally mature male beagle dogs, approximately 24 months of age and weighing 22-28 pounds, were used in this study. All animals were healthy and had fully erupted dentitions. Dogs were chosen for the study because the canine model has been well established as one of the best animal models to examine tooth movement and bone remodeling.[20,80] The housing, care, and experimental protocols were approved by the Institutional Animal Care and Use Committee at Texas A&M University College of Dentistry (IACUC 2016-0214-BCD). To minimize damage to orthodontic appliances, dogs were maintained on a soft food diet throughout the course of the study.

Pre-surgical Preparation

The dogs were quarantined for 10 days, after which time initial records were taken. The animals were fasted for 12 hours and then sedated with an intramuscular injection of ketamine (2.2mg/kg IM) mixed with xylazine (0.22mg/kg IM). Dental prophylaxis was performed using a Cavitron Select ultrasonic scaler (Dentsply, York, PA), irrigated with 0.12% chlorhexidine gluconate. Maxillary and mandibular impressions were taken using alginate in a tray fabricated with Triad custom tray material (Dentsply, York, PA).

Intraoral "scout" periapical radiographs were taken of the right and left side of the mandible using a Planmeca Intra X-Ray unit (Planmeca USA, Roselle, IL) and size 3

film. Bone markers [6 or 8 mm Vector miniscrew implants (Ormco, Orange, CA)] were placed where inter-radicular space was apparent in the scout films. Two miniscrews were placed on each side of the mandible, for a total of four screws per dog. The heads of the miniscrews were removed to the level of the gingiva to decrease likelihood of traumatic failure and animal discomfort.

To facilitate consistent intraoral caliper measurements, notches were drilled in the cusp tips of the mandibular canines and third premolars, and in the most apical portion of the mesiobuccal groove of the mandibular first molar, using a diamond tip bur. To allow radiographic measurements of tooth movement, amalgam tooth markers were placed in the mandibular canines and fourth premolars. A small preparation was made with a round bur and dental amalgam (Dentsply, Milford, DE) was packed into the prepared site and smoothed flush with the tooth surface. Immediately after initial records, dogs were given a single dose of ketoprofen (1mg/kg IM).

Appliance Design

Maxillary and mandibular impressions were poured in die stone. These models were used for fabrication of orthodontic appliances (Figure 1). Appliances were designed based on a previously established protocol.[60,64] Orthodontic band material (Dentauram, Ispringen, Germany) was custom pinched and welded to fit the mandibular canines and third premolars. The interior surface of the band was micro-abraded and small perforations were placed in the bands with a round bur to maximize retention on the tooth.

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Headgear tubes of 0.051" diameter (3M Unitek, Monrovia, CA) were soldered

onto the orthodontic bands on the third premolars. Orthodontic wire of diameter 0.045"

diameter was soldered to the canine bands and inserted through the headgear tubes on

the bands of the third premolars. The wire was designed with a loop at the distolingual

aspect of the canine band to allow for spring attachment at the time of appliance

delivery. A ball of solder was placed on the end of the wire, distal to the third premolar,

for animal comfort. The third premolar tube was able to move freely along the wire. Due

to appliance retention issues, a slight modification of the appliance design was made for

dogs F and G. The extension of the guide wire distal to the third premolar was

lengthened and adapted to the buccal surface of the mandibular first molar. This segment

was bonded with resin cement to the first molar buccal surface at appliance delivery to

increase retention (see Figure 1).

Surgery and Appliance Delivery

For all surgical procedures, the animals were fasted for twelve hours prior to

sedation. Following initial sedation with ketamine and xylazine, dental prophylaxis was

performed using ultrasonic scaler irrigated with 0.12% chlorhexidine gluconate.

Atropine (0.05 mg/kg) was administered prior to intubation to prevent isoflurane-

induced bradycardia. The dogs were then intubated and administered 1.5% isoflurane in

oxygen at a rate of 1 L/minute. Vital signs were monitored throughout procedure. Local

anesthesia (2% lidocaine with 1:100,000 epinephrine) was administered in the surgical

sites via regional infiltration and inferior alveolar nerve block.

Both mandibular second premolars were sectioned, elevated and extracted. In two dogs, the mandibular first premolar root approximated the root of the second premolar, preventing extraction of the second premolar alone. In these dogs, right and left first and second premolars were extracted. Soft tissue was approximated over the extraction sites with simple interrupted 3-0 coated Vicryl resorbable sutures to reduce the risk of post-operative infection.

The experimental surgical side was chosen using an electronically generated random number table. Fiberotomies were performed on the experimental side only using a #15 surgical blade (Integra Miltex, Plainsboro, NJ). The blade was inserted, parallel to the long axis of the tooth, into the gingival sulcus of the mandibular third premolar to the depth of the alveolar crest. The blade was then "walked" circumferentially around the third premolar, maintaining the depth of the blade to the alveolar crest to ensure that all supracrestal fibers were severed. No sutures or surgical dressings were placed. Hemostasis was achieved using gauze wet with sterile saline and held onto the tooth and surrounding tissue with finger pressure for 3-5 minutes.

Once hemostasis was achieved, the teeth were prepped for appliance delivery. Horizontal retention grooves were cut circumferentially around the mandibular canines and third premolars using a round bur, approximately in the middle of the crown. The teeth were micro-abraded, rinsed, and then etched with 37% phosphoric acid gel (Reliance Orthodontic Products, Itasca, IL) for 15 seconds. The etchant was rinsed thoroughly and the teeth were dried. The appliances were then cemented as single units on each side of the mandible with light-cured RelyX Unicem 2 Automix (3M ESPE,

Neuss, Germany) resin cement. Excess cement was removed to prevent gingival irritation.

Appliances were activated by attaching a 6 mm medium nickel titanium coil spring (Ormco, San Dimas, CA) from the third premolar headgear tube attachment to the soldered wire loop on the canine using 0.012" stainless steel ligatures (OSE, Gaithersburg, MD). The springs were activated to 200 g. The force was verified using a Correx force gauge (Haag-Streit, Bern, Switzerland). A small amount of Transbond XT composite resin (Reliance Orthodontic Products, Itasca, IL) was cured over the sharp ends of the steel ligatures to prevent soft tissue irritation.

Intraoral measurements of tooth movement were made to the nearest 0.01 mm using digital calipers (Sylvac, Crissier, Switzerland). The ends of the calipers were inserted into the notches drilled during initial records to measure the distances between the first molar and the canine, the first molar and the third premolar, and the third premolar and the canine. The average of three replicate measurements was used for the analyses.

Post-operative periapical radiographs were taken using a custom radiographic guide assembly (Figure 2), which held a size 4 film extra-orally. The x-rays were emitted from the contra-lateral side at a 45 degree angle. To ensure consistency in exposure distance and angulation, the guide was used to take all subsequent records.

Immediately following surgery, the dogs were given a single dose of Ketaprofen (1 mg/kg IM). They were given antibiotics (Clindamycin 11 mg/kg IM) twice daily for one week after surgery and then on an as needed basis.

Measurements and Appliance Reactivation

Intro-oral caliper measurements and radiographs were taken every two weeks for 8 weeks. At each time point, the coil springs were re-tied and calibrated using the Correx gauge to ensure delivery of 200 g of force. A single investigator (KR) recorded all of the intra-oral measurements.

To evaluate the amount and location of bone modeling, calcein green (10 mg/kg IV) was administered at day 28. Alizarin red (20 mg/kg IV) was administered at day 42 and calcein green was administered a second time at day 53.

Euthanasia

After 8 weeks of tooth movement, the animals were sedated with the previously described ketamine and xylazine mixture. Final records, including periapical radiographs and caliper measurements, were then taken. Appliances were removed by sectioning the orthodontic bands with a diamond bur and peeling the bands away from the premolars and canines.

After surgical plane anesthesia was confirmed, both common carotid arteries were cannulated and the jugular veins were severed. An intracardial injection of 2 cc beuthanasia-D was given. After cessation of heart function was confirmed, approximately 1.5 L of saline followed by 1 L of 4% paraformaldehyde was flushed through the cannulas. The mandible was harvested and stored in 4% paraformaldehyde until samples were collected for histology and μCT.

Radiographic Analysis of Tooth Movement

Periapical radiographs were imported into Viewbox 4.0 (DHAL Software, Kifissia, Greece). Using a custom protocol, all radiographs were analyzed to evaluate translation and tipping of both the mandibular third premolar and the mandibular canine. A single, blinded investigator (KR) digitized all of the radiographs.

The mesial and distal crest of the fourth premolar, the distal crest of the canine, the distal crest of the third incisor, the canine amalgam marker, the midpoint of the canine width at the height of the alveolar bone, the mesial and distal root apices of the third premolar, and the furcation of the third premolar were digitized (Figure 3). A line connecting the distal alveolar crests of the incisor and the fourth premolar was used as the alveolar crest reference line. A line representing the long axis of the canine was drawn from the canine amalgam through the midpoint of the canine width at the height of the alveolar bone. To approximate the long axis of the third premolar, a line was drawn from the root apex midpoint through its furcation. The angles formed between the long axes of the canine and third premolar and the alveolar crest reference line were used to measure tipping of the teeth. The distance between the fourth premolar mesial crest and the furcation of the third premolar, as well as the distance between the furcation of the third premolar and the distal crest of the canine, were used to assess premolar translation.

µCT Assessment of Bone Density

After sacrifice, all specimens were sectioned to include the furcation of the third premolar and the mesial bone into which the third premolar was being moved (Figure 4). These segments were placed into 27 mm wide µCT tubes and stabilized so that the buccal surface was parallel to the long axis of the specimen tubes. The tubes were filled with 0.5% paraformaldehyde and sealed with parafilm (Pechiney Plastic Packaging Company, Chicago, IL). The segments were scanned using the Scanco µCT 35 scanner (ScanCo Medical, Basserdorf, Switzerland) at 30 µm resolution, using 55 kVp, 145 µA and 800 ms integration time. Bone volume fraction and bone density were calculated with Analyze V12.0 software (AnalyzeDirect, Overland Park, KS).

The volume of interest (VOI) selected for analysis included the medullary bone and the lamina dura on the mesial aspect of the mesial root of the third premolar (Figure 4). This VOI was chosen because the premolar is moving in the mesial direction, through this section of bone. From the occlusal view, the lateral limits of the VOI were set at the endosteal surfaces of the buccal and lingual cortices. The distal limit of the VOI was set within the PDL at the mesial aspect of the mesial premolar root to ensure inclusion of the lamina dura. The mesial limit was set by a line perpendicular to the cortices approximately 1 mm away from the lamina dura at its most mesial contour. The middle 60% of the mesial root length in an apical-incisal direction was utilized in the analysis. It was determined by locating the coronal cross-section where the alveolar crest became apparent and the cross-section where the root apex ended and removing the 20% of slices at each end. The resulting volumes were approximately 4 mm in height. The

bone volume to total volume ratio and bone density were both measured for the volume of interest.

Histologic and Fluorescent Image Evaluation of Specimens

Three randomly selected specimens were evaluated using hematoxylin and eosin (H+E) staining. The specimens were decalcified in ethylenediaminetetraacetic acid, dehydrated in graded alcohol, cleared with xylene, then infiltrated and embedded in paraffin. Specimens were cut along the sagittal plane to thicknesses of 5 to 6 μm. Starting with the section closest to the buccal cortical surface, every 15th to 20th section was selected. There were a total of approximately 10 sections per tooth that were mounted. These sections were mounted onto glass slides and stained with hematoxylin and eosin. Images of the slides were captured at 2.5x, 5x or 10x magnification and digitized using an Olympus VS120 Virtual Slide Scanner (Olympus Scientific Solutions Americas Corp., Waltham, Massachusetts).

The three undecalcified specimens were analyzed by fluorescent microscopy to evaluate new bone formation. Specimens were dehydrated in graded alcohols, followed by acetone and then methyl methacrylate. They were embedded in methyl methacrylate until complete polymerization occurred. Specimens were sectioned into segments approximately 125 μm in thickness. Two of the specimens were cut along the sagittal plane, starting from the buccal cortical surface. One specimen was cut along the coronal plane, starting at the level of the CEJ. Sections were then ground and polished to a final thickness of approximately 75 μm. Alizarin red and calcein green images of the sections

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were captured at 5x magnification and digitized using an Eclipse 80i confocal

microscope (Nikon, Tokyo, Japan).

Statistical Analyses

All statistical analyses were performed using IBM SPSS® version 23 (IBM

Corp., Armonk, NY). Tooth movement and μCT data were normally distributed and

described by means and standard deviations. Non-parametric tests were used due to

small sample size. Side differences were evaluated using Wilcoxon signed ranks test.

CHAPTER III

RESULTS

After surgery, healing proceeded normally without any signs of infection at the extraction or fiberotomy sites. During the course of the experiment, the bands of three dogs loosened due to either bond failure or breakage (Table 1). All three dogs experienced damage on both the control and experimental sides. All appliances were replaced or repaired within 72 hours of detecting the damage. Tooth movements and bone quality of Dog E were not evaluated due to an excessive number of appliance bond failures and breakages.

Tooth Movements

Intraoral caliper measurements showed that the distance between the third premolar and the canine decreased by 2.31 ± 0.62 mm on the experimental side, and 2.08 ± 0.56 mm on the control side (Table 2, Figure 5). These changes represent statistically significant movements from their initial positions, but the between-side differences were not consistent or statistically significant at any of the time points. The distances between the first molars and the third premolars increased significantly (1.08 ± 0.41 mm on the control side and 1.16 ± 0.27 mm on the experimental side), but the between-side differences were not statistically significant (Figure 6).

The radiographic measurements taken at the height of the alveolar bone from fourth premolar to third premolar showed 0.78 ± 0.29 mm and 0.82 ± 0.44 mm of mesial

tooth movement on the control and experimental sides, respectively (Table 2, Figure 7).

The between-side difference was not statistically significant at any of the time points.

The third premolar tipped significantly in both groups, with the control premolar tipping

mesially 3.82 ± 3.49° and the experimental premolar tipping mesially 5.62 ± 1.04°

(Table 2). The between-side differences were not statistically significant at any time

point (Figure 8).

µCT and Histologic Assessments

µCT analysis showed that the volume fraction of bone mesial to the third

premolar was slightly higher on the control than experimental side (Table 3). The control

bone volume fraction was 0.63 ± 0.18, while the experimental side bone volume fraction

was 0.60 ± 0.20, with no statistically significant difference between sides (p=0.600,

Figure 9). While the apparent density was slightly higher on the control (601.14 ±

153.26 mg HA/cm^3) than the experimental (580.60 ± 166.00 mg HA/cm^3) side, and the

material density was slightly higher on the experimental (895.55 ± 25.12 mg HA/cm^3)

than the control (892.02 ± 17.96 mg HA/cm^3) side, neither of the differences were

statistically significant (Figure 10). There also were no statistically significant between-

side differences in trabecular number, trabecular thickness or trabecular spacing (Table

3).

The H + E sections of the sulcus mesial to the third premolar showed intact soft

tissue attachment of the marginal gingiva to the tooth root surface on both the control

(Figure 11) and experimental sides (Figure 12). On the control side, large fiber bundles

were seen fanning out from the root surface to the marginal gingiva and the alveolar crest. In the experimental sections, some of the small fibers appeared to show re-organization back to the cementum, but the large, highly-organized bundles were not evident.

The H + E sections of the bone mesial to the third premolar showed conflicting results with respect to osteoclastic activity and PDL space. Either the experimental side showed a slightly wider PDL space and more osteoclasts on the surface of the bone mesial to the tooth being moved than the control side (Figures 13 and 14), or there were no differences between the control and experimental sides (Figures 15 and 16). On this basis, there was no consistent pattern of differences between control and experimental sides noted.

Fluorescent imaging showed new bone formation on the trailing edge of the mesial root of the third premolar on both sides (Figure 17). The green labels show bone mineralization when the calcein was administered at week 4 of tooth movement and again 2-3 days prior to sacrifice; the red label shows bone that was mineralizing at week 6 when alizarin red was administered. Labeled bone was not present on the leading edge of the mesial root of the third premolar on either side. There was no obvious between-side differences between the three animals included in fluorescent analysis.

CHAPTER IV

DISCUSSION

Circumferential fiberotomies alone do not increase rates of tooth movement. In the present study, there were no statistically significant increases in tooth movement on the CSF side, and there was no consistent pattern of change indicating that there might be. Previous animal studies evaluating the effects of CSF on tooth movements have generally indicated faster experimental than control tooth movement. Glenn et al[76] reported an 108% increase in canine retraction in cats following fiberotomy. Tuncay et al[77] and Young et al,[78] who studied the effects of CSF on tooth movement in rats, found that fiberotomy increased tooth movements by 23% and 74%, respectively. The differences between these studies and the current study are likely due in part to the magnitude of orthodontic forces used relative to the experimental animal body size. For example, Tuncay et al[77] applied 25 g of force to a rat incisor, a force comparable to over 1000 g in a human. The amount of surgical insult produced in previous animal studies could also have played a role in increasing tooth movements after fiberotomy. Using scalpel blades in smaller animals (rat or cat) might be expected to cause relatively more tissue trauma than in larger animals (dogs). This is important because increased tooth movements have been directly related to the amount of osseous and soft tissue damage produced during surgery.[63,64,81] Lastly, the previous CSF studies may have overestimated tooth movements because they did not differentiate between bodily tooth movement and

tipping. In the current study, premolar tipping was limited and not statistically significant.

Fiberotomy alone does not significantly increase tooth movement because it does not cause the necessary changes in the bone adjacent to the tooth, i.e. it does not induce the RAP effect. μCT analyses in the current study showed that the bone adjacent to the experimental root through which the tooth was being moved was basically the same, in terms of density, volume fraction and maturation, as the control bone. Whenever increases in tooth movement have been reported, they have been associated with decreased bone density and bone volume fraction.[64,82,83] In contrast, when surgical interventions had no effect on the density and volume of bone through which the teeth must move, rates of movement were not affected.[60,62]

It is likely that CSF has only a slight and temporary effect on adjacent bone. Hoffman, who evaluated bone of dogs 2 and 4 weeks after CSF surgery, found significantly decreased bone volume fraction in the coronal most 20% of the lamina dura and alveolar bone adjacent to the tooth root.[84] However, the differences in bone volume fraction did not extend to bone in the more apical region along the tooth root. Moreover, newly formed bone was noted 4 weeks post-fiberotomy, and osteoclast activity, which was greater on the experimental than control side at 2 weeks, was no longer significantly different on the experimental than control side. This transient change in bone remodeling could cause an increase in initial tipping immediately following fiberotomy, which could explain why previous animal studies found increases in tooth movement after performing fiberotomy procedures at regular intervals.[76-78] Van Gemert et al[85] also

reported transient and limited changes in bone density after micro-osteoperforation (MOP), with differences of less than 5% between experimental and control bone 1.5mm from the site of bone damage. These limited effects explain why multiple MOPs performed in bone adjacent to teeth do not cause significant increases in tooth movement.[62] As such, a single fiberotomy (which is not directly damaging the bone like MOPs), as performed in the current study, should not be expected to cause bony changes large or extensive enough to significantly affect tooth movement.

The fluorescent evaluations performed in the current study showed similar patterns of new bone formation on both control and experimental sides, which further supports the lack of difference in tooth movements between sides. No previous study has performed fluorescent evaluations of bone formation after fiberotomy. In the current study, fluorescent markers were administered at 4 weeks, 6 weeks and 7.5 weeks after CSF. Since there were no differences in experimental and control sides, any changes in bone remodeling that occur following fiberotomy must occur within the first 4 weeks after surgery.

Possible explanations regarding the changes in the PDL and adjacent bone following surgical disruption of the marginal gingiva, like those that occur after fiberotomy, have been suggested. One hypothesis proposes that damage to the junctional epithelium causes proteins to be expressed in the epithelial rests of Malassez (ERM), which may affect calcium binding and deposition.[70] Changes in protein expression have been shown in ERM throughout the PDL and into the furcation,[86] but bony changes associated with this protein expression have not previously been studied. The results of

the current study do not support the idea of a diffuse reaction in the bone after gingival fiberotomy. Another hypothesis suggests that fibroblasts comprising the supracrestal fibers undergo a strain relaxation when severed and release signals that initiate a cascade of inflammatory mediators that stimulate soft tissue and bone resorption.[66] One inflammatory mediator, P2X4, has been shown to increase specifically in the marginal gingiva and coronal PDL.[67] Results of the current study support a more limited, site-specific, effect on adjacent bone.

While supracrestal fibers reattach shortly after CSF, they remain disorganized over the short term. The current study showed that supracrestal fibers were attached directly to the root surface eight weeks after severing their attachments. However, the fibers were not as thick or as highly organized as those in the control specimens. Glenn et al[76] reported fiber reattachment on both sides of teeth being moved as early as one week after fiberotomy in cats, with fibers on the tension side oriented parallel to tooth movement and fibers on the pressure side oriented perpendicular to tooth movement. When teeth are not being moved, fiber attachment 2 and 4 weeks after fiberotomy has been reported, with the fibers being less organized on the CSF than control side.[84] It is likely that fibers re-attach within 1-2 weeks following fiberotomy and start re-organizing by 8 weeks, but a time frame for complete re-organization has yet to be established. It is possible that previous animal studies performing CSF at regular intervals prevented fiber re-attachment, which could have increased the effects on tooth movement.

Clinical Implications

Clinically significant faster tooth movements are not likely to occur after a single fiberotomy procedure. Changes in the bone after fiberotomy are too localized and short-lived to affect rates of tooth movement. The available literature indicates that the location of bone resorption achieved by fiberotomy may increase the likelihood of tooth tipping. More research is needed to determine the effects of multiple fiberotomy procedures on tipping in humans and larger animals Additionally, the long-term effects on soft tissue attachment and crestal bone height have yet to be determined. As such, fiberotomies performed with the intent of increasing rates of tooth movement in patients cannot be presently supported.

CHAPTER V

CONCLUSIONS

1. Circumferential fiberotomies alone do not increase the rate of tooth movement.

2. There is no difference in bone density or maturity 8 weeks post-fiberotomy. In other words, fiberotomy does not induce the regional acceleratory phenomenon.

3. Fibers are re-attached to the tooth root surface by 8 weeks post-fiberotomy, but not completely re-organized.

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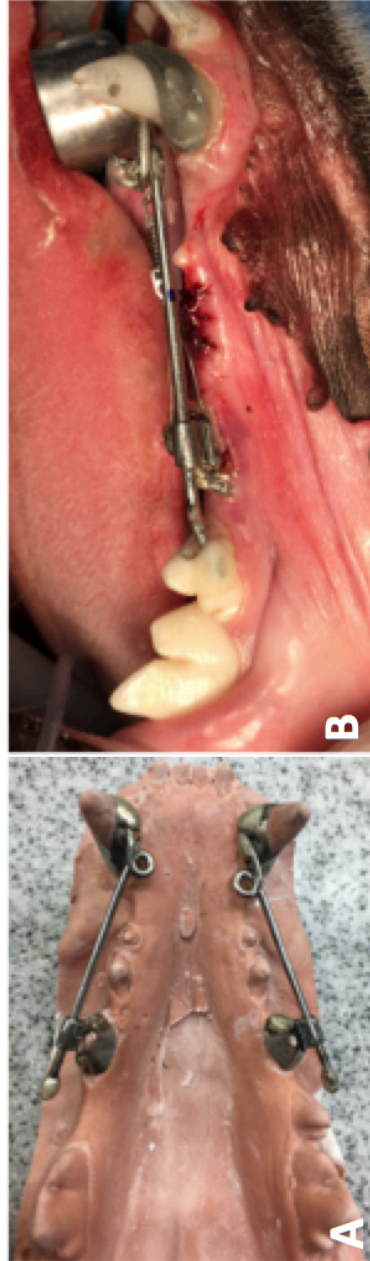
FIGURES

Figure 1: Appliance design and placement. A) appliances shown on model. B) appliance delivery showing slight modification of design for Dogs F and G.
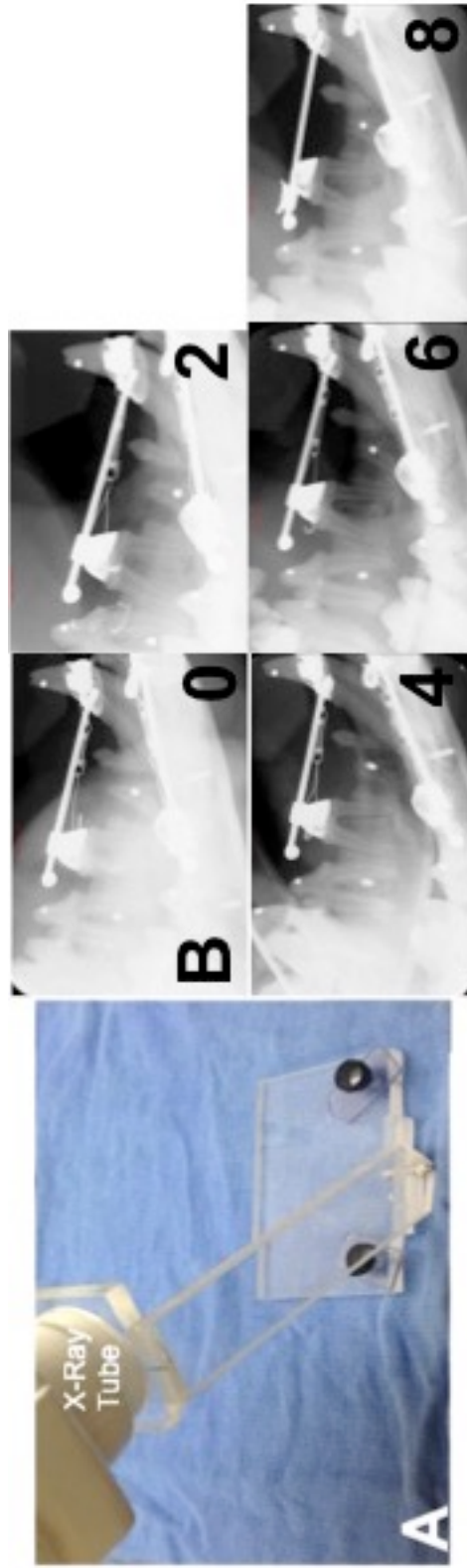
Figure 2. Custom radiographic guide. A) Radiographic guide to maintain consistent angulation and distance. B) Representative series of radiographs for one animal, week of records indicated in lower right of each image.
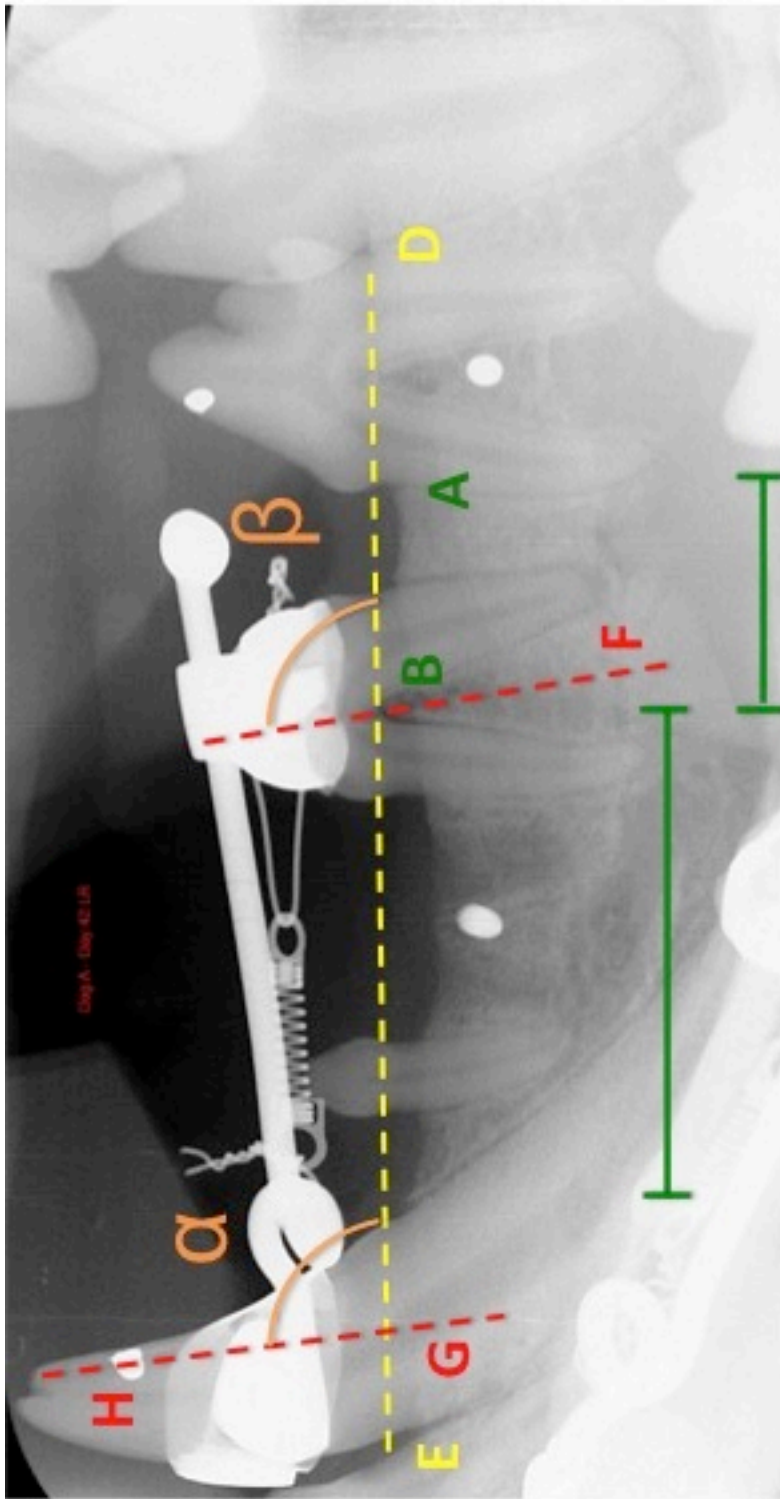
Figure 3: Radiographic landmarks and measurements. Green: A – mesial crest of forth premolar, B – furcation of third premolar, C – distal crest of the canine. Yellow: D – distal crest of forth premolar, E – distal crest of incisor, dotted line from D-E = alveolar crest reference line. Red: F – midpoint of mesial and distal root apices of third premolar, G – midpoint of canine width at height of alveolar bone, H – canine amalgam marker, dotted line from F-B = long axis of third premolar, dotted line connecting G-H = long axis of canine. Orange: α – canine angulation, β – third premolar angulation. Distances from A-B and B-C were used to assess third premolar translation.
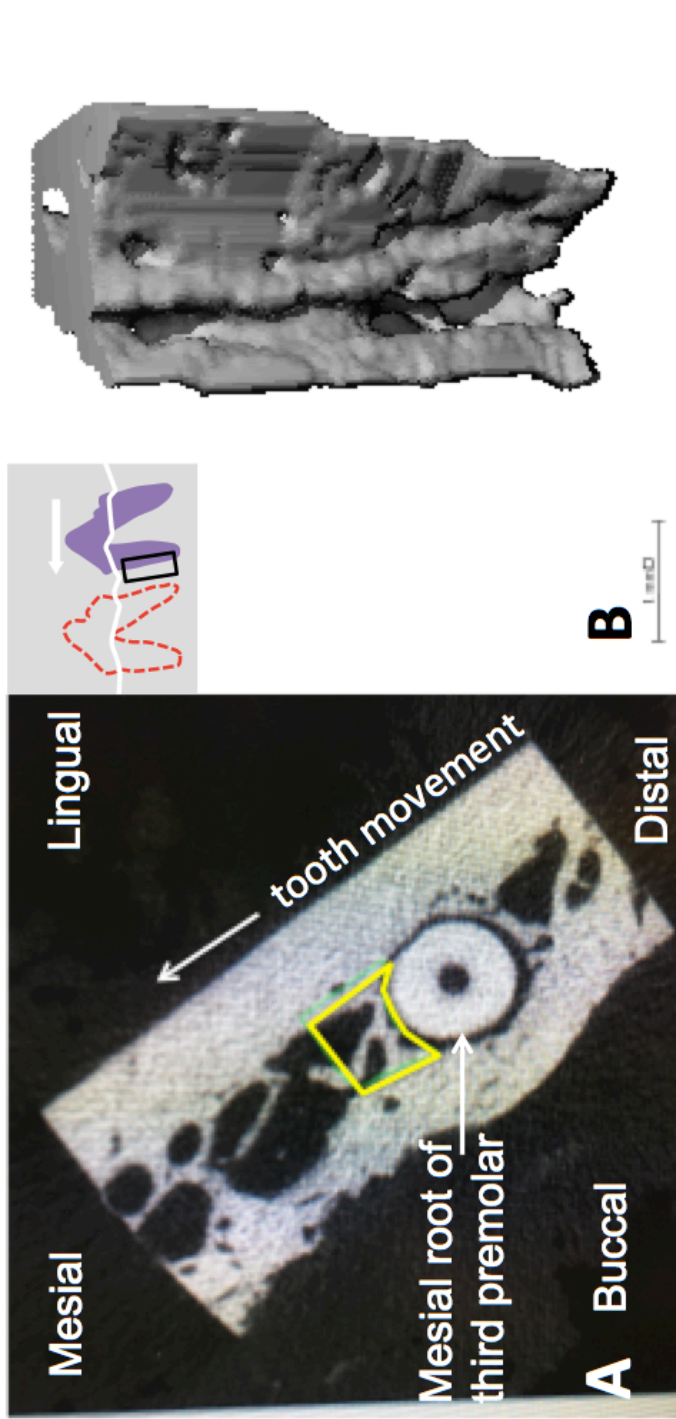
Figure 4: μCT volume of interest. A) 2D image of the bone segment used for μCT analysis, with the volume of interest outlined in yellow, B) 3D depiction of the volume of interest (scale = 1mm) which represents the bone adjacent to the middle 60% of the total third premolar root length.
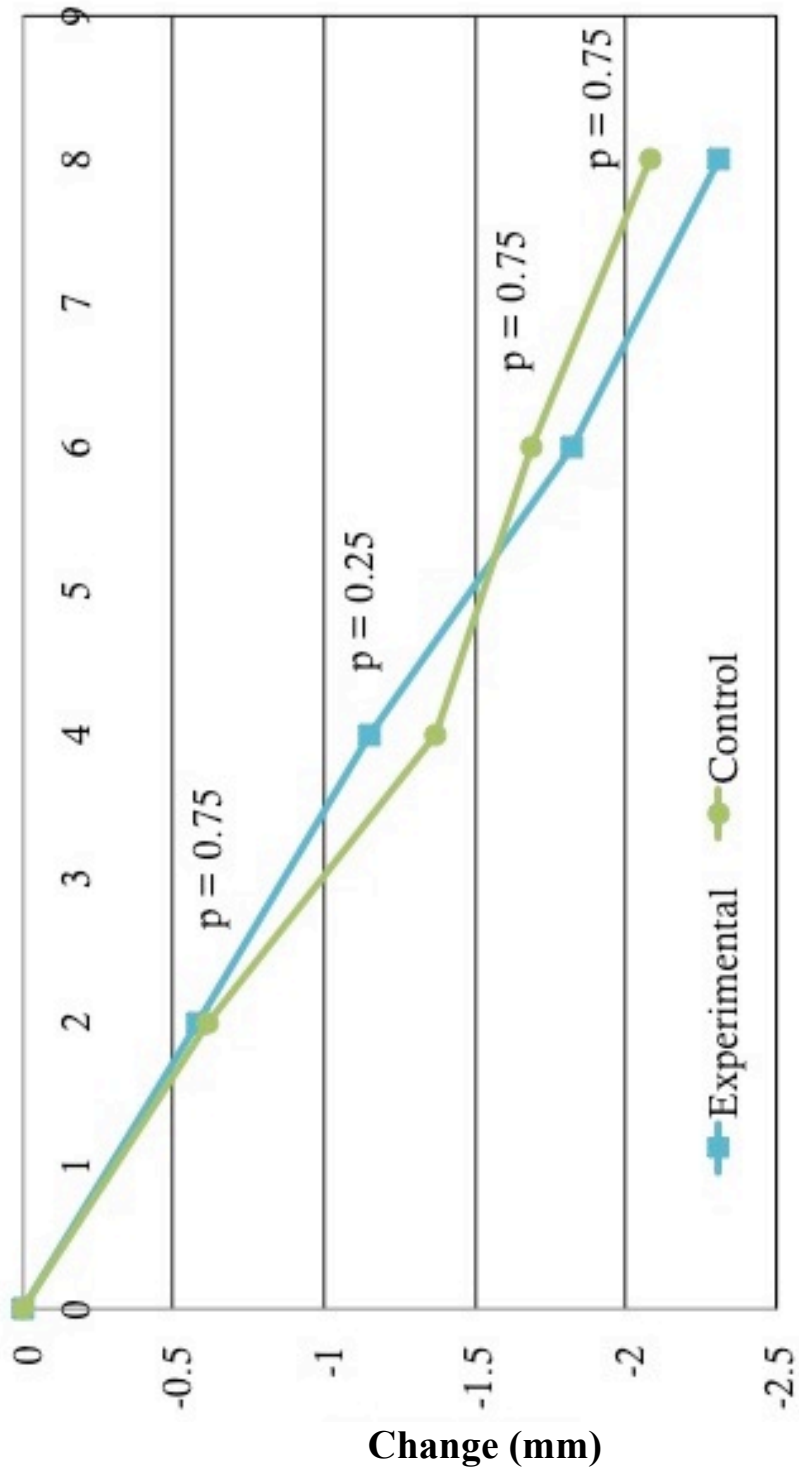
Figure 5: Intraoral caliper measurements of P3-C space closure.
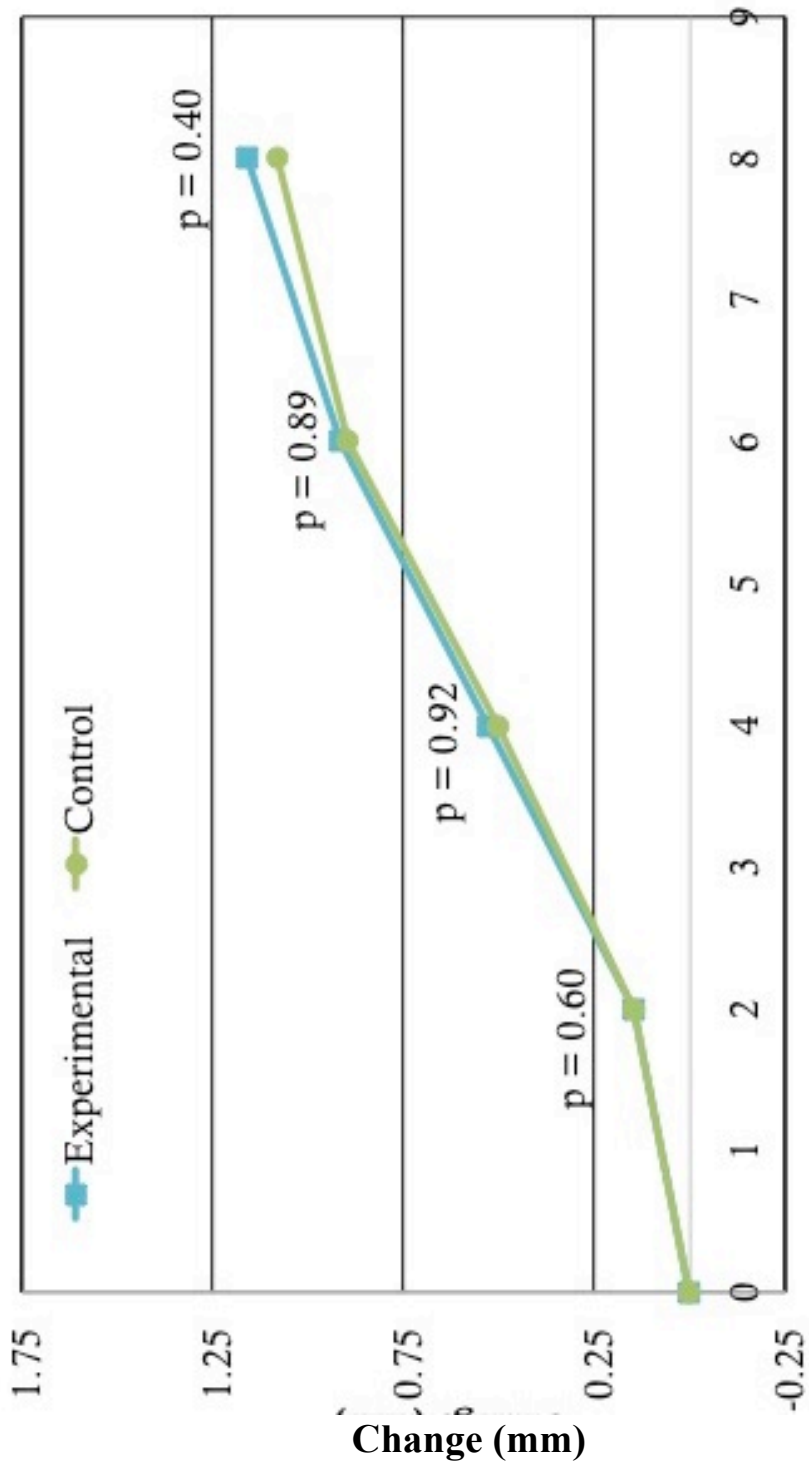
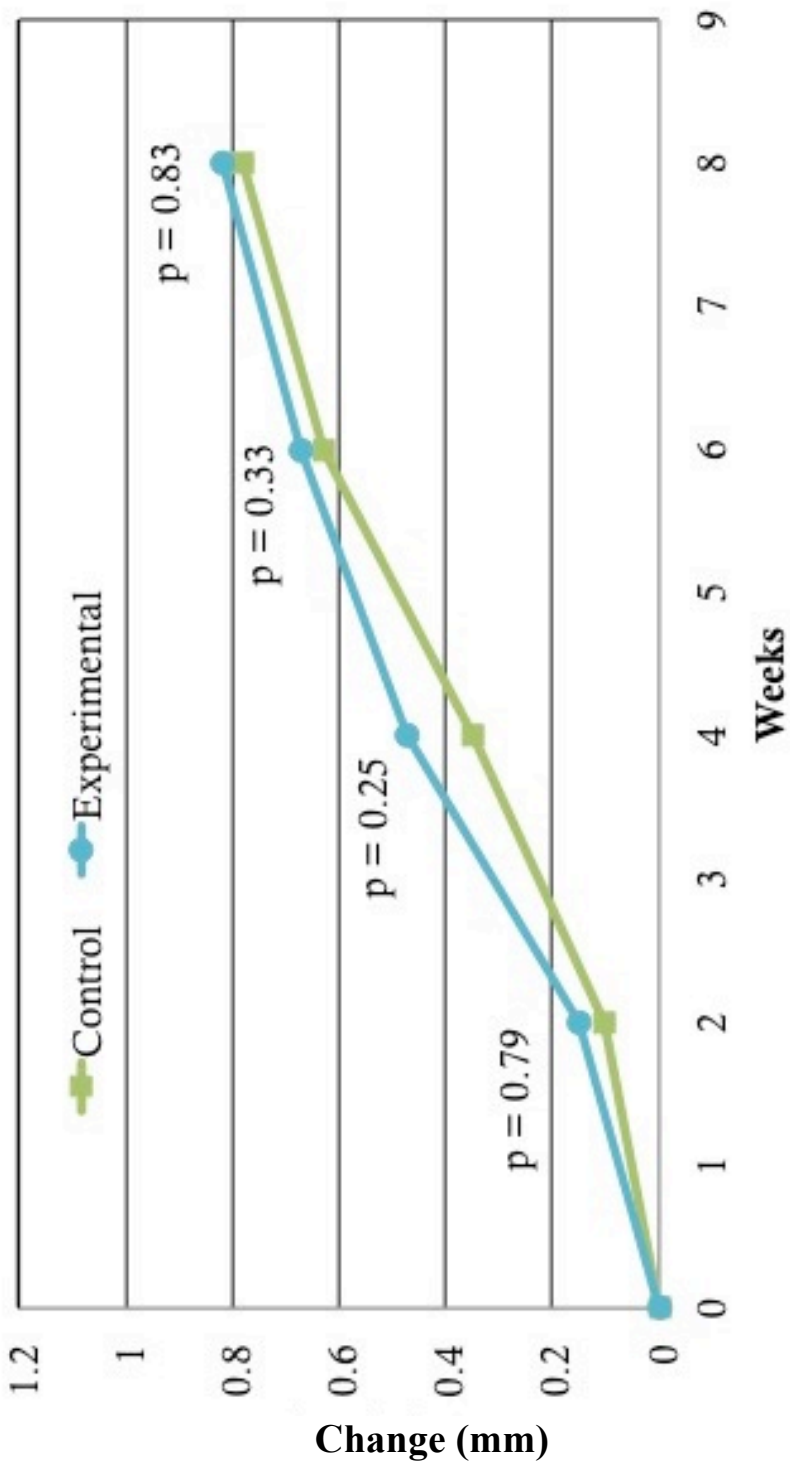**Figure 6:** Intraoral caliper measurements of M-P3 space opening.
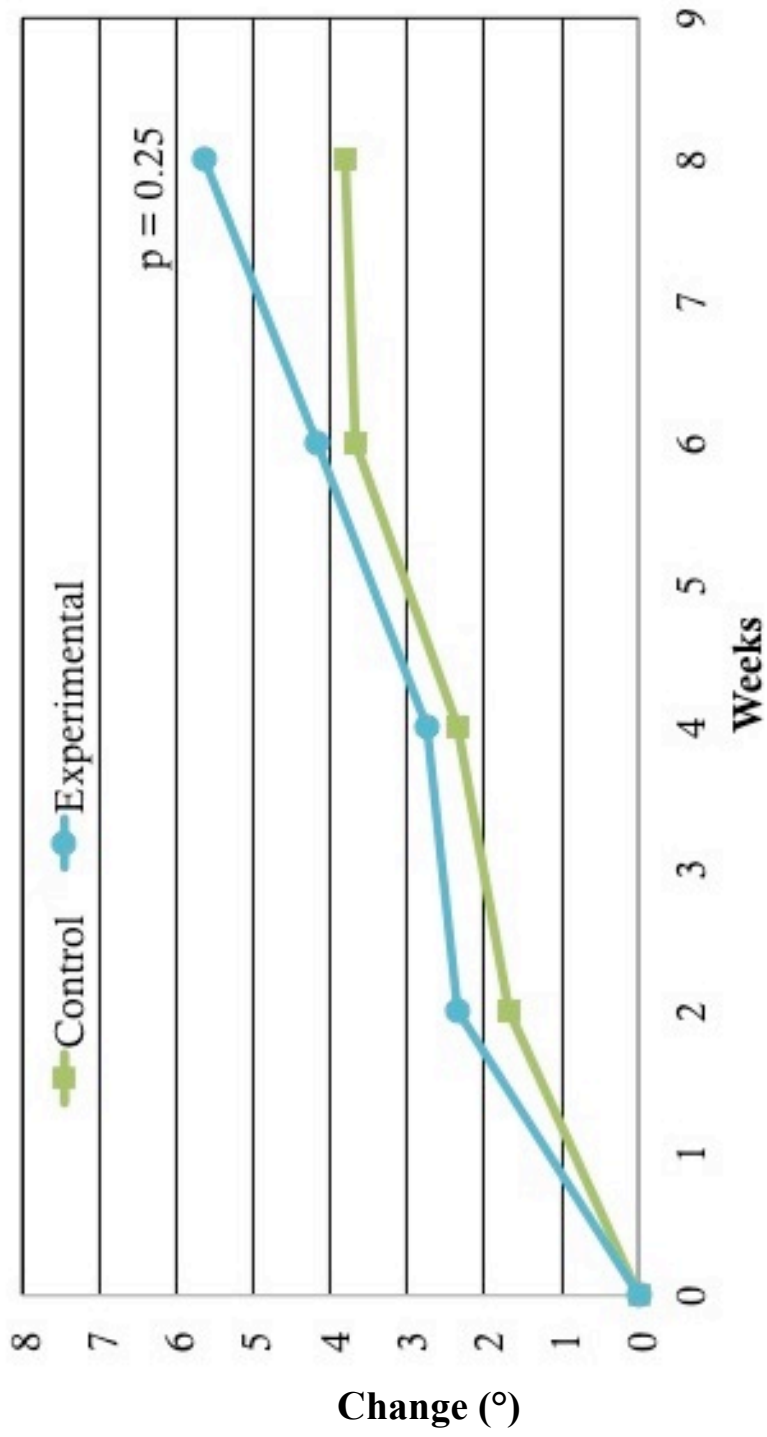
Figure 7: Radiographic measurements of P4-P3 space opening.

Figure 8: Radiographic measurements of third premolar tipping.
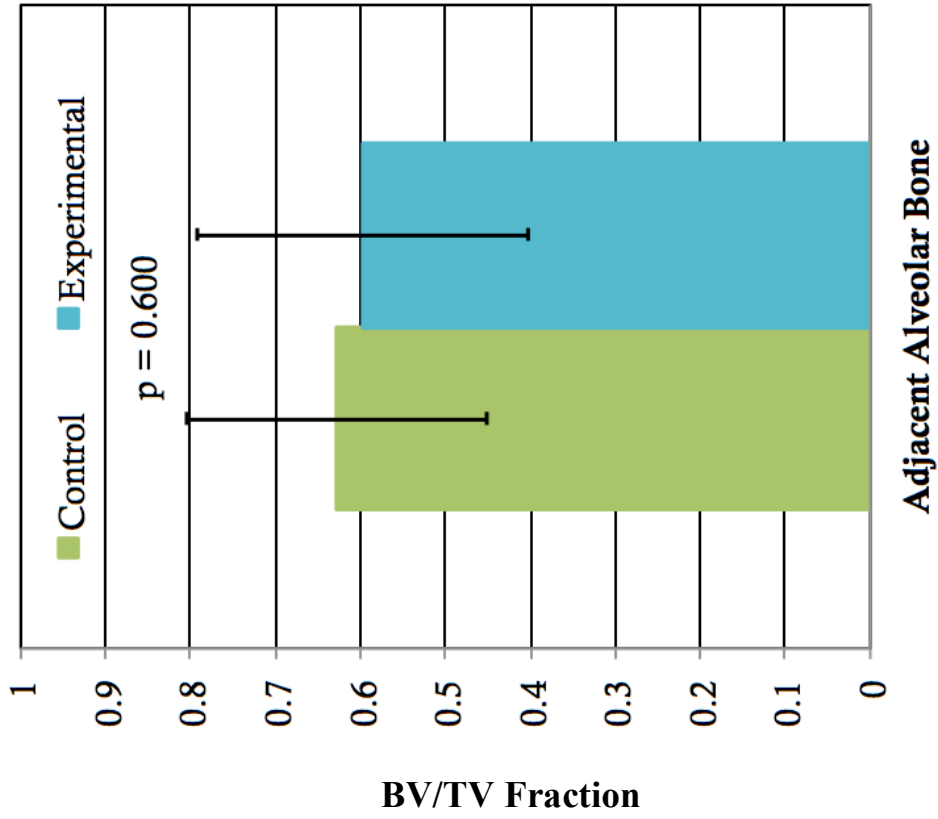
Figure 9: Adjacent alveolar bone volume fraction. Volume fraction (BV/TV) of alveolar bone mesial to third premolars, along with standard deviation and the probability (p) of between-side difference.
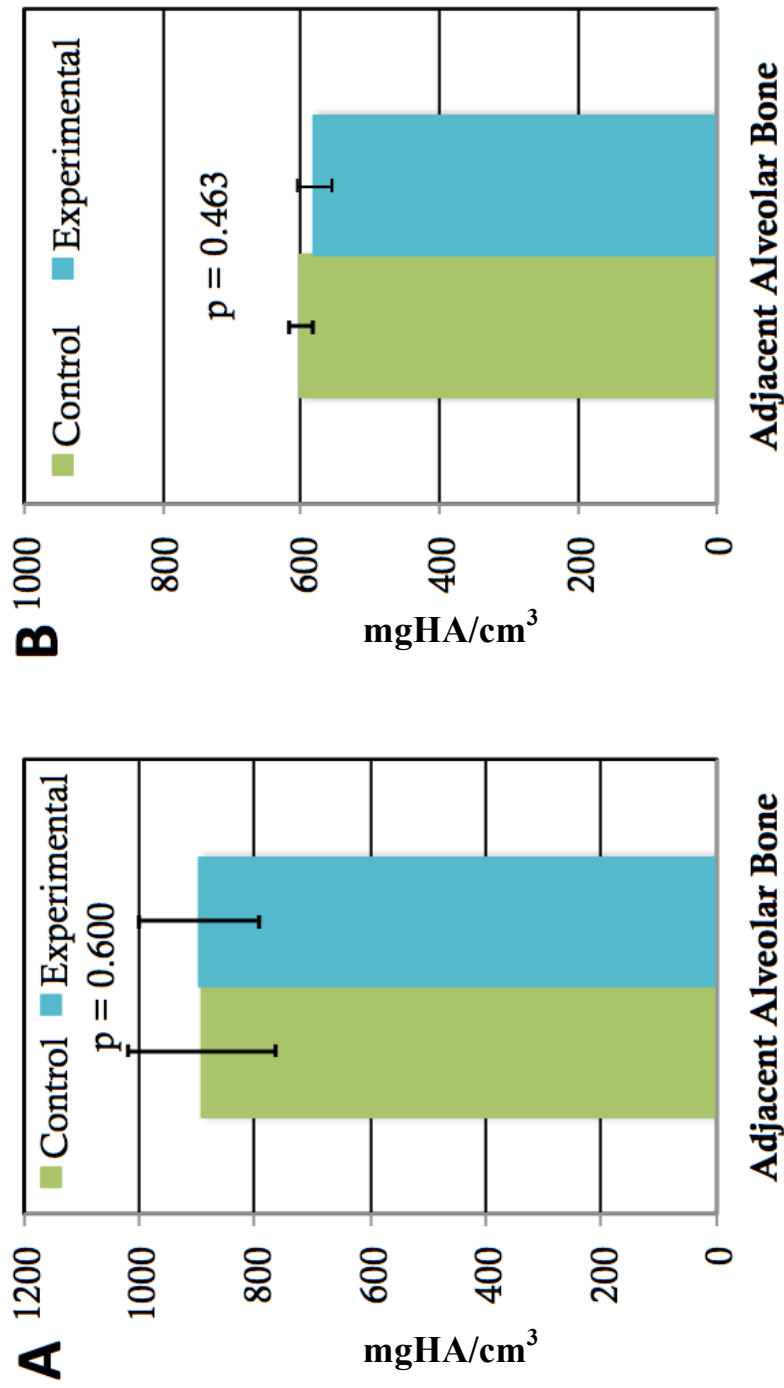
Figure 10: Adjacent alveolar bone density. A) Material and B) apparent density of the alveolar bone mesial to the third premolars along with standard deviation and the probability (p) of between-side differences.
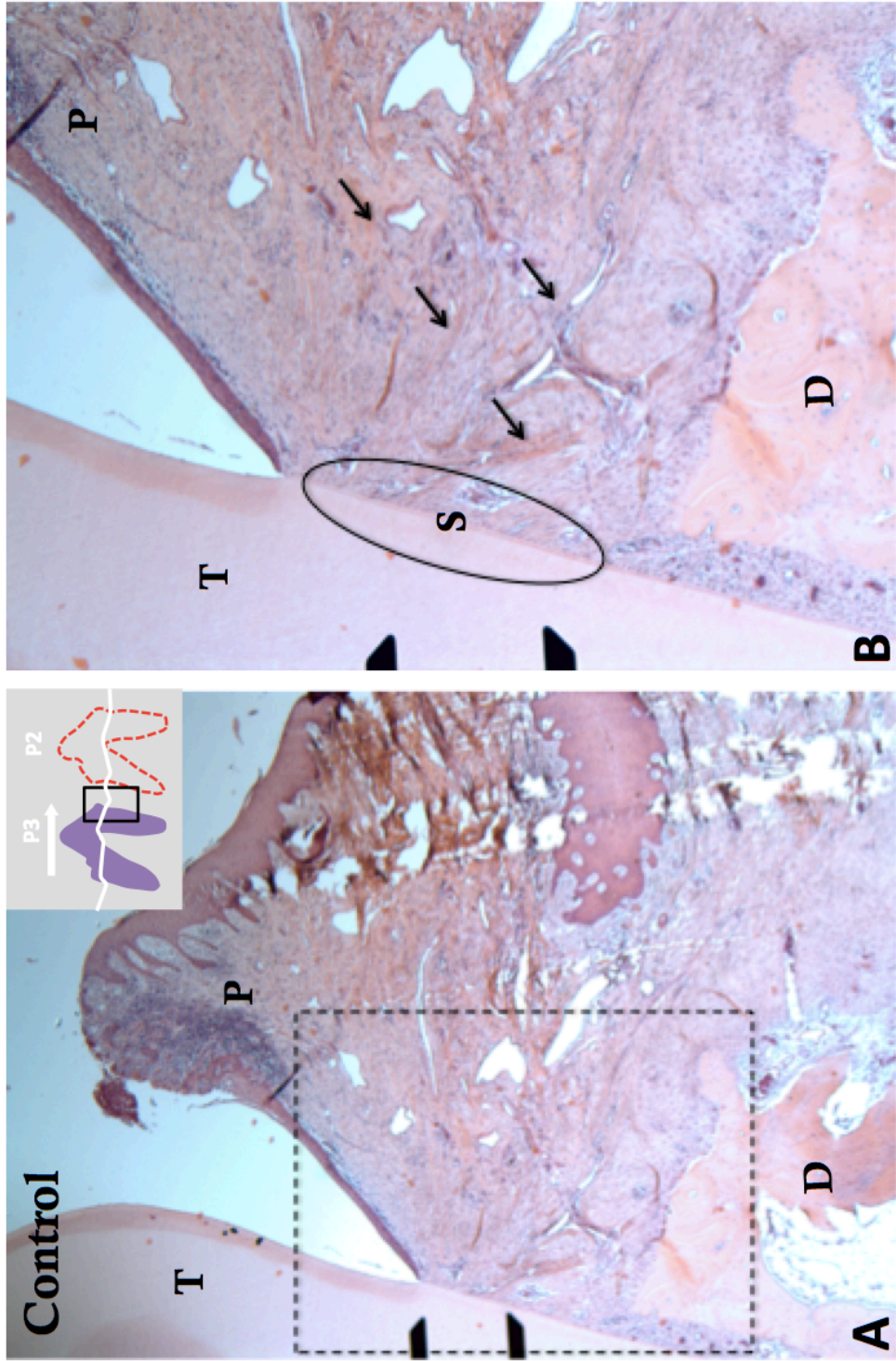
Figure 11: Control soft tissue H&E evaluation. H + E slides depicting the soft tissue mesial to the control third premolar from one animal at A) 2.5x magnification (caliper = 250 µm) and B) 5x magnification (caliper = 125µm). T - third premolar, S – cellular attachment of soft tissue to cementum, P – papilla, D – alveolar bone crest. Black arrows represent fiber bundles.
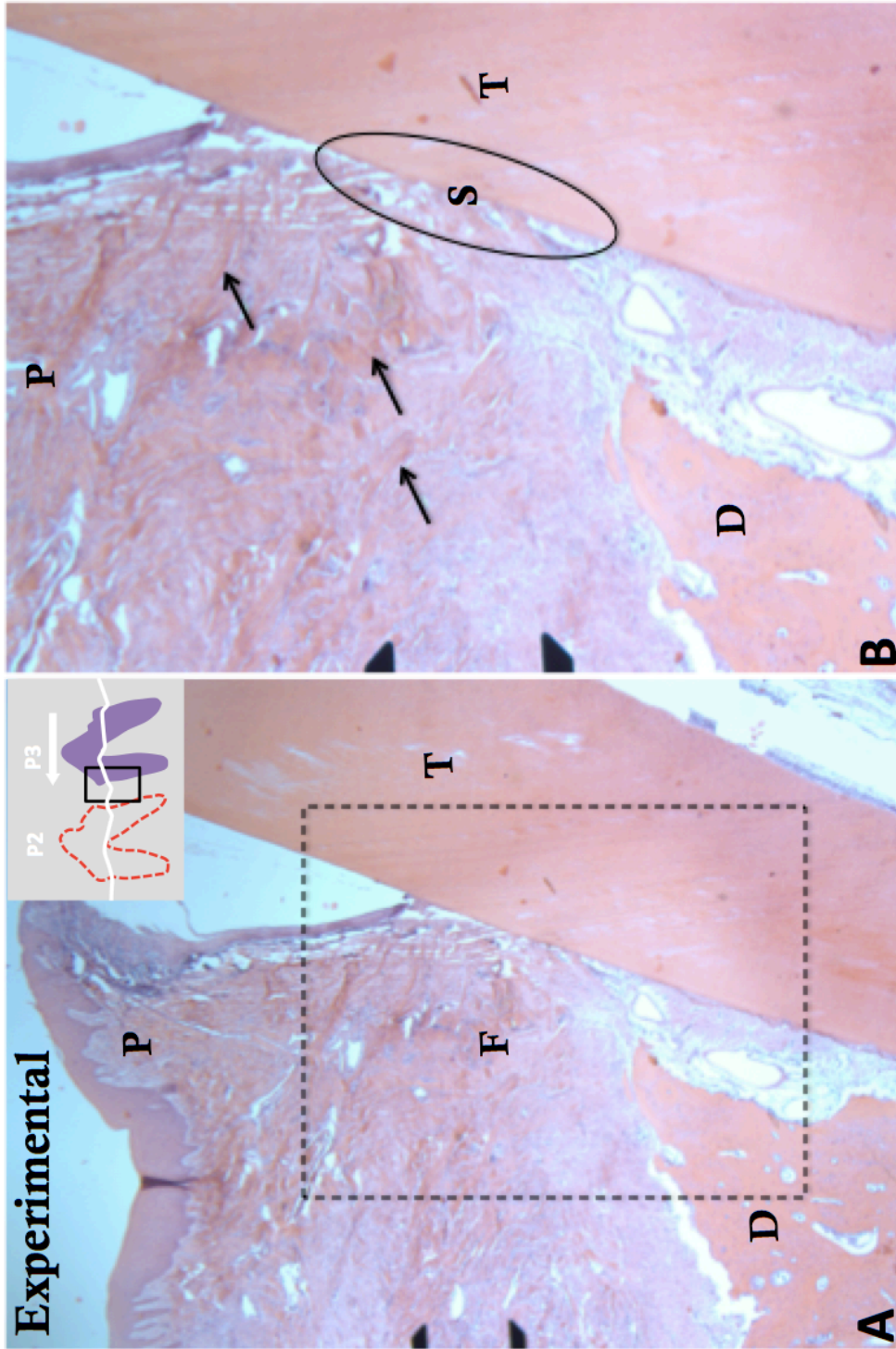
Figure 12: Experimental soft tissue H&E evaluation. H + E slides depicting the soft tissue mesial to the experimental third premolar from one animal at A) 2.5x magnification (caliper = 250 μm) and B) 5x magnification (caliper = 125μm). T - third premolar, S – cellular attachment of soft tissue to cementum, P – papilla, D – alveolar bone crest. Black arrows represent collagen fibers.
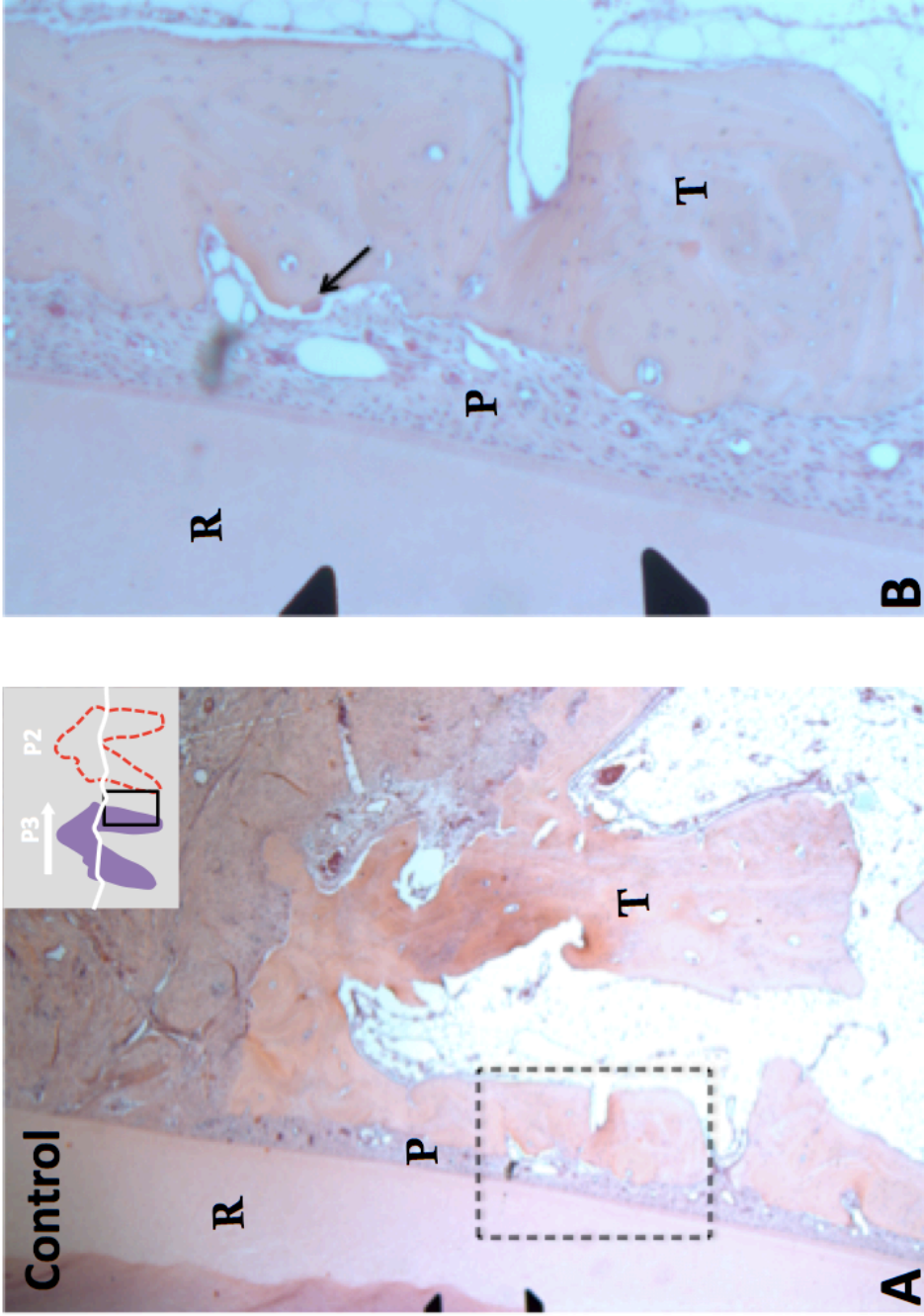
Figure 13: Control hard tissue H&E evaluation. H + E slides depicting the root surface, PDL space and bone mesial to the control third premolar from one animal at A) 2.5x magnification and B) 10x magnification. R - third premolar root, P – periodontal ligament, T – trabecular bone. Black arrow indicates osteoclast.
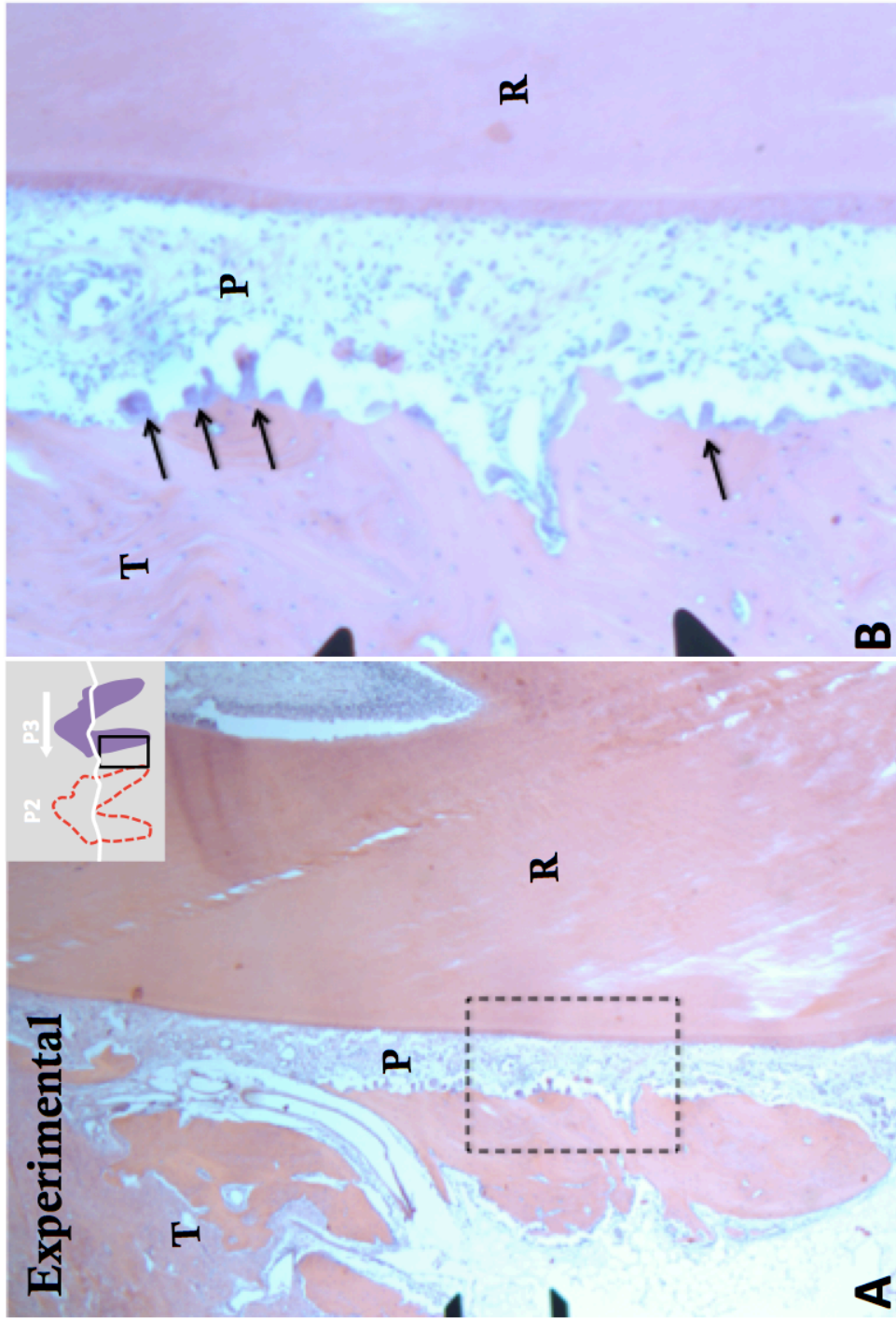
Figure 14: Experimental hard tissue H&E evaluation. H + E slides depicting the root surface, PDL space and bone mesial to the experimental third premolar from one animal at A) 2.5x magnification (caliper = 250 μm) and B) 10x magnification (caliper = 62.5μm). R - third premolar root, P – periodontal ligament, T – trabecular bone. Black arrows indicates osteoclasts.
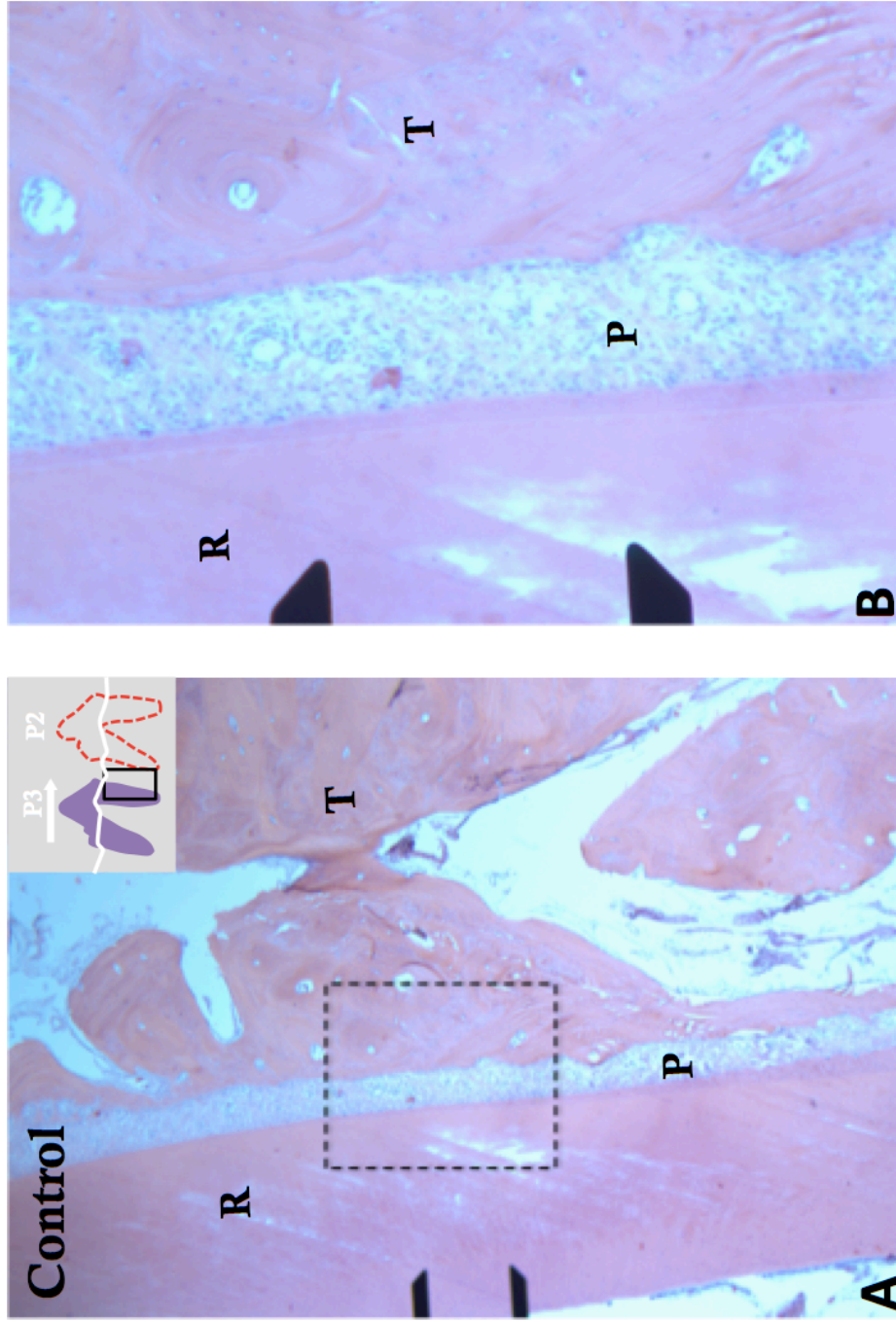
Figure 15: Control hard tissue H&E evaluation, animal #2. H + E slides depicting the root surface, PDL space and bone mesial to the control third premolar from one animal at A) 2.5x (caliper = 250 μm) and B) 10x magnification (caliper = 62.5μm). R - third premolar root, P – periodontal ligament, T – trabecular bone.
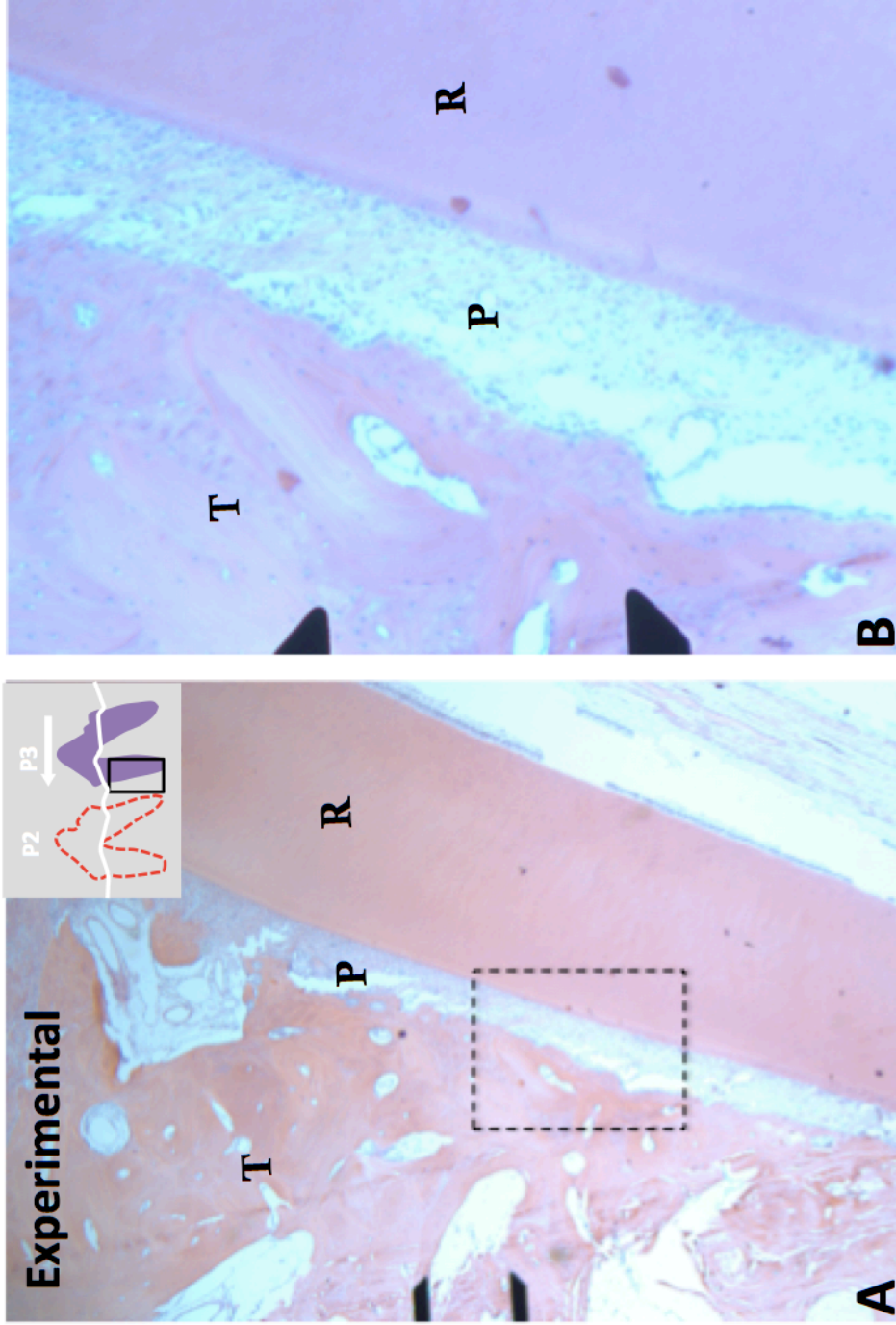
Figure 16: Experimental hard tissue H&E evaluation, animal #2. H + E slides depicting the root surface, PDL space and bone mesial to the experimental third premolar from one animal at A) 2.5x magnification (caliper = 250 μm) and B) 10x magnification (caliper = 62.5μm). R - third premolar root, P – periodontal ligament, T – trabecular bone.

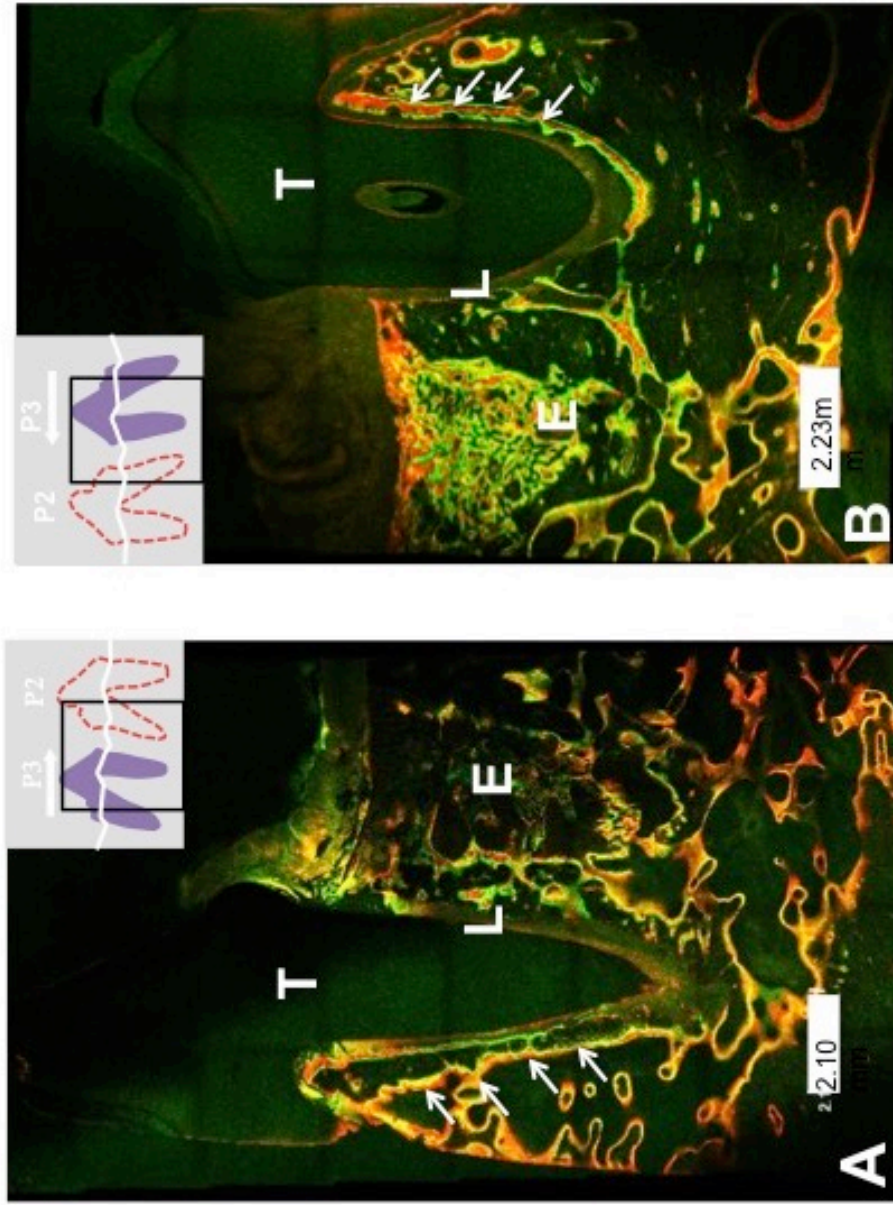**Figure 17**: Fluorescent images constructed using confocal microscope. A) control third premolar (scale = 2.10 mm), B) experimental third premolar (scale = 2.23 mm). Green shows calcein-labeled bone, red shows alizarin-labeled bone. T – third premolar, E – extraction site of second premolar, L – leading edge of mesial root. White arrows indicate newly formed inter-radicular bone on the trailing side of the mesial roots.

Table 1: Adverse events related to appliances.

Dog	Day	Description	Impact	Action
C	21	Experimental side third premolar band loose	Up to 48 hours without force	Rebonded band same day
C	42	Control side third premolar band loose and guide wire bent	Between 48-72 hours without force	Remade appliance and replaced on day 44
C	56	Control side third premolar band loose	Up to 48 hours without force	Rebonded band same day
D	70	Control and Experimental third premolar bands were loose	Up to 96 hours without force	Nothing because damage was detected day of sacrifice
E	3	Control side third premolar band loose and guide wire bent	Up to 96 hours without force	Remade appliance and replaced on day 6
E	9	Control side third premolar band loose	Up to 48 hours without force	Rebonded band same day
E	17	Experimental side third premolar band loose and guide wire bent	Up to 96 hours without force	Remade appliance and replaced on day 20
E	25	Control side appliance debonded completely	Up to 24 hours without force	Made final records and removed experimental appliance, did not replace appliances

Table 2. Tooth movement and tipping measurements of teeth on the experimental and control sides over 8 weeks of tooth movement.

	Experimental		Control		Difference
	Mean	S.D.	Mean	S.D.	Prob
Intraoral					
P3-C (mm)	**-2.31**	0.62	**-2.08**	0.56	0.75
M-P3 (mm)	**1.16**	0.27	**1.08**	0.41	0.40
Radiographic					
P4-P3 (mm)	**0.82**	0.44	**0.78**	0.29	0.83
P3-C (mm)	**-0.95**	0.67	**-0.93**	0.48	0.68
P3 Angle (°)	**5.62**	1.04	**3.82**	3.49	0.25
C Angle (°)	**1.68**	1.32	0.28	3.50	0.46

Bold = Statistically significant (p<0.05) within-side differences

Table 3. MicroCT analysis of bone adjacent to third premolar in the direction of tooth movement on the experimental and control sides after 8 week experimental period.

	Experimental		Control		Difference
	Mean	SD	Mean	SD	Prob
Bone Volume Fraction (bone volume/total volume)	0.60	0.20	0.68	0.18	0.600
Apparent Density (mg HA/cm^3)	580.60	166.00	601.14	153.26	0.463
Material Density (mg HA/cm^3)	895.55	25.12	892.02	17.96	0.600
Trabecular Number (1/mm)	1.55	0.11	1.64	.19	0.116
Trabecular Thickness (mm)	0.40	0.13	0.39	0.10	0.753
Trabecular Spacing (mm)	0.25	0.14	0.23	0.15	0.463