THE EFFECT OF INCREASED CORTICAL DAMAGE ON BONE DENSITY AND BONE TURNOVER IN MATURE RABBITS: A RANDOMIZED SPLIT-BODY STUDY

A Thesis

by

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ABSTRACT

The purpose of this study was to investigate the biological changes in bone density and bone modeling of the cortical bone when increasing the amount of cortical damage.

Using a split body design, rabbit tibias were randomly allocated into a control group with one osteoperforation (OP) or experimental group with four OPs through the cortical bone. Two weeks prior to sacrifice. μCT, Vickers Hardness microindentation, and histologic analyses were performed.

The experimental bone was significantly less dense than the control bone up to 2.0 mm away from the OPs after 2 weeks of healing. The Vickers hardness of the control bone was significantly harder than the experimental bone up to 3.3 mm away from the OPs. The H&E sections of cortical bone showed greater amounts of woven bone within the OP site in the control specimen than in the experimental specimen after 2 weeks of healing while the experimental group had a greater amount of unmineralized fibrous tissue within the OP site. Added tissue was found on the periosteal and endosteal surfaces in both samples. The control group primarily had added woven bone while the experimental group primarily had added fibrous tissue, indicating an earlier stage of healing in the group with four OPs. There were areas of acellularity directly adjacent to the OPs in almost all of the control and experimental samples, which extended up to 0.875 mm away from the OPs. TRAP staining indicated greater osteoclastic activity in the experimental group than the control group up to 1.0 mm away from the OPs.

In conclusion, greater cortical damage resulted in adjacent cortical bone that was less dense, less hard and had a higher degree of osteoclastic activity. The rate of healing was also slower with greater damage.

DEDICATION

This work is dedicated to my family, especially my parents, Alvin and Sally Garcia, and to my husband John Sugay without whom all of this would not be possible. Thank you for your unwavering love, encouragement and support.

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INTRODUCTION AND LITERATURE REVIEW

OVERVIEW

According to the American Association of Orthodontists, the average length of comprehensive orthodontic treatment is 22 months[1], but treatment times can last up to 36 months for patients with severe orthodontic problems.[1-5] For nonextraction cases, treatment durations range from 21-27 months, while extraction treatment ranges from 25-35 months.[1,3-7] Extended treatment time has been associated with an increased rate of root resorption,[8-12] decalcification and caries,[11,13] periodontal problems[14,15] and increased costs to the orthodontist and patient. As orthodontists, it is important to consider methods to decrease treatment time in order to minimize these risks while still achieving satisfactory results. One way to reduce treatment time is to increase the rate of tooth movement.

Currently, performing corticotomies is the best-established surgical intervention used to accelerate tooth movement by inducing the regional acceleratory phenomenon (RAP).[16] The RAP is a biological process that causes a temporary increase of soft and hard tissue remodeling with a rise in cellular activity and bone remodeling at the site of injury through the recruitment of osteoblasts and osteoclasts.[16] It involves an increase in perfusion, an increase in bone turnover, and a decrease in bone mineral density. The Wilcko brothers in 2001 hypothesized that corticotomies induced the RAP, a biological rather than mechanical process that aids in increasing the rate of tooth movement.[17-19] The corticotomy procedure as originally described by the Wilcko brothers, which is also

known as periodontally accelerated osteogenic orthodontics (PAOO), selective alveolar decortication (SAD), or surgically accelerated osteogenic orthodontics (SAOO), involves laying full-thickness mucoperiosteal flaps labially and lingually and creating perforations into the cortical bone surrounding the teeth to be moved.[20,21] Particulate bone grafting material is then placed over the alveolar bone and the flaps are sutured back together. There is considerable variation in the surgical techniques used depending on the surgeon's preference.

More recently, there has been a trend toward less invasive methods to create bone damage and induce the RAP. Surgical techniques that do not require reflection of the gingival tissues have been popularized due to the decrease in trauma to the patient and the potential to preserve periodontal support. Dibart was the first to introduce flapless alveolar decortication using a piezosurgery unit and termed the procedure "piezocision."[22] Subsequent studies have evaluated the efficacy of flapless corticotomies on tooth movement using various instruments such as surgical blades[23-25], carbide burs[25,26], bone awls[27] and the PROPEL device[28,29]. The PROPEL device creates micro-osteoperforations (MOPs) by threading a 1.4 mm diameter stainless steel mini-screw implant through the gingiva and into the bone. The assumption that many of these studies make is that any amount of injury to the bone can induce the RAP and accelerate tooth movement. However, there have been limited studies evaluating the effect of greater and lesser damage to the bone.

There has only been one study that has been designed to evaluate the effect of greater and lesser surgical insult on bone. Cohen et al[30] used a split-mouth design in

foxhounds to compare the effects of two surgical techniques on tooth movement. The surgical insult on the first group (RAP group) involved extraction of the maxillary first premolars, removal of the inter-septal bone within 1 mm of the second premolars and creation of buccal and lingual vertical grooves within the extraction socket. In the second group (RAP+ group), the maxillary first premolars were also extracted and the inter-septal bone removed 1 mm from the second premolars. A full thickness mucoperiosteal flap was laid and the buccal plate between the second premolar and canine was removed. Osteotomies were then performed extending to, but not through, the lingual cortex 1 mm distal and apical to the second premolar. This study found that the increased surgical trauma, and therefore, an increase in RAP, increased the rate and ultimately the amount of tooth movement. A companion study found that there was significantly less bone on the RAP+ side compared to the controls and it was also less dense and less mature.[31] Histologic evaluations indicated an increased number of osteoclasts and greater bone surface areas on the RAP+ side than on the RAP side. These two studies combined demonstrated that greater surgical insults produced a larger RAP response, characterized by less dense and less mature bone, and these changes were made possible by increased osteoclastic activity. These changes translated to greater amounts of tooth movement. The problem with this study design was that different surgeries were done to induce the increase in trauma. Not only were corticotomies performed, but they were combined with extractions and in some cases removal of the cortical plate. It is difficult to prove with this study design that it was the increase in

cortical damage that facilitated the increase in tooth movement rather than the different surgeries performed.

No study, to date, has quantified the effects of increasing cortical damage on the cortical bone adjacent to the surgical sites in a controlled manner that a split-body design can provide. The purpose of the present study was to investigate the biological changes in bone density and bone modeling of the cortical bone when increasing the amount of cortical damage.

LITERATURE REVIEW

The basic problem in orthodontics is the speed of tooth movement. However, before evaluating ways to increase the rate of tooth movement, the normal rate of tooth movement and biology of tooth movement must be understood. There are many factors that can influence the rate of tooth movement, such as the magnitude of force, duration of force and the mode of force application. It is particularly important to understand how bone biology affects tooth movement. For example, studies have shown correlations between tooth movement and bone remodeling and bone density. Teeth move faster when there is an increase in bone turnover and a decrease in bone density. A number of non-surgical approaches will be reviewed that have been shown to increase the rate of tooth movement. For example, using pharmacologic agents such as Vitamin D3, osteocalcin and prostaglandin E1 through their various mechanisms could theoretically increase the rate of tooth movement. Similarly, application of pulsed electromagnetic fields and direct electric currents have been studied. However, these methods had

problems associated with them that led clinicians to consider surgical intervention such as inducing damage to the bone. The review will then focus on corticotomies, which increase the rate of tooth movement by taking advantage of the RAP. The RAP is the body's normal response to bone injury and is limited in duration and the effect. It lasts about two to three months in humans. During this time, the bone displays transient osteopenia and a decrease in bone density, which allows teeth to move more rapidly through the bone. There is indirect evidence evaluating the association between the amount of surgical insult and more rapid tooth movement by comparing studies combining extractions and corticotomies with studies looking at corticotomies alone. It will be shown that only one study has been designed to evaluate the effect of greater and lesser surgical insult on bone. The problem with this study is that different surgeries were done to induce the increase in trauma. Not only were corticotomies performed, but they were combined with extractions and in some cases removal of the cortical plate. It is difficult to prove with this study design that it was the increase in cortical damage that facilitated the increase in tooth movement rather than the different surgeries performed. To date, there has been no controlled study that looks at the effects on the bone turnover and bone density of adjacent cortical bone with an increase in cortical damage.

Normal Orthodontic Tooth Movements

Research quantifying the rate of tooth movement has shown that teeth move approximately 1 mm per month.[32-37] The rate of tooth movement achieved is largely dependent upon the amount of force applied and ultimately has a biologic limit. Typical

forces used in orthodontic treatment range from 60 to over 400 grams. However, more force does not always translate into faster tooth movement, due to an increase in hyalinization and the undermining resorption that results. Other factors that have been thought to be associated with the rate of tooth movement include the mode of force application[33-36,38], continuity of force application[37], and the influence of friction and binding between brackets and archwires.[39-41]

Boester and Johnson[42] conducted a split-mouth study on patients who required the removal of four first premolars and distal retraction of the canines. Each quadrant was randomly allocated to different retraction springs producing 2, 5, 8, and 11 oz of force, which are equal to approximately 55, 140, 225 and 310 g of force, respectively. The appliances were activated weekly for a total of ten weeks. Study models and lateral cephalograms were used to measure the amount of space closure after ten weeks. The data showed that the 2 oz force level produced significantly less movement than 5, 8 and 11 oz. There was no significant difference between 5-11 oz in terms of the amount of space closure. The authors postulated that this is possibly due to the fact that in the 5-11 oz range, bone resorption appears to be occurring at a maximal rate and may constitute the rate-limiting factor.

Iwasaki et al[32] conducted a split-mouth study retracting maxillary canines into first premolar extraction spaces using closing loops activated with NiTi closed coil springs. A continuous retraction force averaging 18 g (0.63 oz) on one side was compared to 60 g (2.12 oz) on the other side. A Nance or Nance/TPA to the first molars and coligation of the second premolars to the second molars bilaterally was used as

anchorage to maximize canine retraction and minimize molar protraction. Their results showed that distal movement of the canines was 0.87 and 1.27 mm/month for the 18 and 60 g of retraction forces, respectively. The authors found that although the 60 g force produced greater distal movement of the canine, the difference was mainly due to the large amount of tooth movement that occurred during the first three days of the study. After the first three days, the rates of tooth movement were not significantly different between force magnitudes.

In addition to the magnitude of force, the mode of force application is also important. Samuels et al[38] compared the rate of space closure after a premolar extraction using a nickel-titanium (NiTi) closed coil spring and an elastic retraction module using sliding mechanics on a 0.019 x 0.025-inch stainless steel arch wire in a 0.022 x 0.028-inch pre-adjusted stainless steel bracket slot. The NiTi closed coil spring produced approximately 150 g of force compared to 400 to 450 g of force produced with elastomeric ligation. Seventeen subjects had the two quadrants of each arch randomly assigned to receive each space closure method. Study models were then analyzed to determine the average rate of tooth movement. The results showed that 105 g NiTi closing coils closed space on average 0.26 mm per week, which translates to 1.04 mm per month. Elastomeric chain closed space approximately 0.19 mm per week, which translates to 0.76 mm per month. In a follow-up study by Samuels et al[33], the same sliding mechanics were used to compare 100 g to 200 g NiTi closing coils. They found that the 100 g springs closed space at a rate of 0.16 mm per week or 0.64 mm per month and the 200 g springs closed space at a rate of 0.24 mm per week or 0.96 mm per month.

The results were combined with the previous study to compare the three springs and the elastic module. They concluded that the 150 g and 200 g springs produced similar rates of space closure, faster than the elastic module and the 100 g spring.

Bokas et al[34] conducted a similar study evaluating maxillary canine retraction and molar anchorage loss. They compared a NiTi spring to elastomeric chains using sliding edgewise mechanics. Twelve patients who required maxillary canine retraction into first premolar extraction sites were used in a split-mouth design, with each subject serving as their own control. The NiTi springs and elastomeric chains were all precalibrated to deliver approximately 200 g of force and were reactivated at 28-day intervals. Maxillary study models were taken to evaluate space closure. The study found that the mean rate of space closure with NiTi springs was 1.85 mm/month, which was only slightly greater than the elastomeric chains (1.68 mm/month). The mean rates of anchorage loss for each technique were not statistically different.

Nightingale and Jones[35] also compared the rates of space closure with elastomeric chain and NiTi coil springs. They performed a split-mouth study of canine retraction based on 22 patients treated with pre-adjusted edgewise appliances and using sliding mechanics on 0.019 x 0.015-inch posted stainless steel arch wires. The elastomeric chain delivered an initial force of 70-450 g, with a mean of 209 g, while the NiTi coils produced an initial force of 150-460 g, with a mean of 300 g. Similar to the study by Samuels et al[33], space closure with elastomeric chains occurred at a rate of 0.21mm per week or 0.84 mm per month while space closure with NiTi coil springs

occurred at a rate of 0.26 mm per week or 1.04 mm per month. The rates were not significantly different.

Keng et al[36] conducted a split-mouth randomized controlled clinical trial to evaluate the rate of space closure and tooth angulation using pre-activated T-loops fabricated from titanium-molybdenum alloy (TMA) and nickel-titanium (NiTi). Twelve patients who had upper premolars extracted were used. Each quadrant in the maxilla was randomly allocated to either TMA or NiTi T-loops. The loops were activated 3 mm per visit to deliver a load of approximately 150 g to the upper canines. Study models were taken each month to measure the amount of tooth movement. The results showed a mean rate of canine retraction of 0.91 mm/month and 0.87 mm/month for NiTi and TMA T-loops, respectively. This study showed that there was no difference in the rate of space closure when using pre-activated TMA or NiTi T-loops to retract upper canines.

It is also important to note that there is a difference in tooth movement between intermittent and continuous force applications. In a split-mouth study by Daskalogiannakis and McLachlan,[37] the amount of maxillary canine retraction was compared using constant force versus an impulsive force of short duration. TMA vertical loops were used on both sides of the maxilla, with rare earth block magnets applying a continuous force of 60 g on one side. On the other side, a force of 70 g that rapidly declined after initial activation was re-activated every 6 weeks. The rate of tooth movement on both sides was compared over a period of 3 months. The results showed a rate of 0.63 mm per month on the intermittent force side versus 1.22 mm per month on

the continuous force side. The study concluded that a greater rate of tooth movement could be achieved with continuous force application.

Friction and binding between brackets and arch wires can also affect the rate of tooth movement when using sliding mechanics, but the evidence is controversial. Scott et al[39] performed a randomized controlled trial comparing patients with self-ligating brackets to conventional brackets. They found no difference in efficiency of initial alignment. Similarly, da Costa Monini et al[40] performed a split-mouth study comparing self-ligating and conventional brackets and found no difference in the rate of canine retraction. However, Burrow et al[41] conducted a split-mouth study comparing self-ligating brackets (Damon3 and SmartClip) and conventional twin brackets (Victory Series). Their study found a statistically significant difference in the rate of canine retraction over a four-week period, with conventional brackets having more retraction than the self-ligating brackets. They hypothesized that the self-ligating brackets had a narrower width that could have caused more binding within the bracket slot.

Biology of Tooth Movement

To better understand how tooth movement rates might best be increased, it is important to understand the biology that dictates the biologic limits. Tooth movement within the alveolar process requires bone resorption in the area where the teeth are being moved into and bone formation in the sites from where teeth are moving away. King et al,[43] who analyzed the alveolar bone adjacent to molars being moved orthodontically in rats, found that bone resorption was found primarily on the “pressure” side or the side

teeth are being moved towards and that bone formation was found on the "tension" side or the side teeth are being moved away from.

There are many proposed mechanisms to explain bone remodeling and tooth movement. These include the piezoelectric, mechanotransduction and pressure-tension theories among others. These hypotheses differ in their explanation of tooth movement and it is possible that the actual method of tooth movement may be a combination of these theories.[44]

Piezoelectric Hypothesis

When an applied force deforms bone, a change in electric polarity along the surface of the bone occurs. This phenomenon is termed the piezoelectric effect and can be seen in many crystalline structures.[45] Bassett and Becker[46] in the early 1960s found that the crystalline structures in bone under mechanical stress respond to deformation by emitting electrical potentials that can stimulate bone formation. During tooth movement, there is a net movement of positively charged ions to the pressure side and negatively charged ions to the tension side. This may be thought to play a role in signaling tooth movement. The phenomenon is brief, and upon removal of the force the equivalent signals are generated in the opposite direction.[45] Most researchers believe that the piezoelectric theory itself is insufficient to explain tooth movement alone.

Pressure-Tension Hypothesis

Research by Sandstedt[47], Oppenheim[48], and Schwarz[49] in the early 20th century led to development of the pressure-tension theory. This theory postulates that movement of the tooth leads to stretching and compression of the periodontal ligament (PDL). On the

tension side, bone deposition can be seen on the alveolar wall and on the pressure side, bone resorption can be seen. There is also a narrower PDL space on the pressure side. Under light pressure, osteoclasts can remove bone immediately adjacent to the PDL that is under compression in a process called frontal resorption. Under high pressures, however, the development of a cell-free necrotic zone of hyalinization is seen. Osteoclasts must then invade from undamaged medullary bone through the site of hyalinization in a process known as undermining resorption.

Mechanotransduction Hypothesis

The possibility of electrical currents stimulating bone remodeling might be better explained by the mechanotransduction hypothesis. The mechanical energy of orthodontic force is thought to be converted into an electrical and/or biochemical signal by osteocytes in the bone.[50] It is thought that the signals for bone remodeling are relayed through the network of canaliculi and gap junctions in bone, in what is referred to as the osseous connected cellular network.[51] Through osteocyte communication and interaction, there is an increase in bone remodeling. Therefore when force is applied, movement of teeth occurs.

The correlation between rates of tooth movement and rates of bone remodeling has been shown by Verna et al. Verna et al[52] used 52 rats divided into three groups: control, high bone turnover and low bone turnover, to study the influence of bone metabolism on the rate of tooth movement. The high and low bone turnover groups were induced pharmacologically by changes in thyroid function for four weeks prior to the application of orthodontic force. A mesially directed constant force was applied to the

maxillary first molar for three weeks and rates of tooth movement were recorded. Compared to the control group, there was a significantly higher rate of tooth movement in the high bone turnover group and a reduced rate of tooth movement in the low bone turnover group.

Similarly, Midgett at al[53] induced nutritional hyperparathyroidism in beagle dogs and compared tooth movement and bone metabolism to a control group. They found more rapid tooth movement in hyperparathyroid animals. These animals also had significantly decreased bone density, as well as bone remodeling changes consistent with high PTH levels. Their findings suggest that tooth movement is dependent upon the state of calcium metabolism in alveolar bone.

Bone density also affects the rate of tooth movement, with faster tooth movement occurring in less dense bone and slower tooth movement occurring in more dense bone. This was shown by Goldie et al[54] in a study using 35 rats divided into a control group and a calcium-deficient group. The maxillary molars were orthodontically moved mesially and tooth movement was evaluated. They found that the animals with decreased bone density had a significant increase in tooth movement compared to the control group. It concluded that the accelerated tooth movement was correlated to the decreased bone density in the calcium-deficient rats. Similarly, Ashcraft et al[55] showed the effects of osteoporosis on tooth movement using 16 New Zealand white rabbits. Experimental rabbits received cortisone acetate to induce osteoporosis while control rabbits received no pharmacologic intervention. The maxillary left first molars were

mesialized orthodontically. The rabbits with cortisone-induced osteoporosis exhibited a 3-4-fold increase in the rate of tooth movement compared to the controls.

Accelerating the Biology of Tooth Movement

As discussed above, decreases in bone density and increases in bone remodeling are associated with faster rates of tooth movement. Administration of pharmacologic agents to induce these effects and increase tooth movement has been studied by Collins and Sinclair[56], who evaluated the effect of localized injections of Vitamin D in cats, Kobayashi et al[57] who looked at the effect of local administration of osteocalcin in rats, Yamasaki et al[58,59], who administered prostaglandin E1 in monkeys and humans., Li et al[60] and Soma et al[61,62] who investigated the effects of parathyroid hormone in rats and Madan et al[63] and McGorray et al[64] who administered human relaxin hormone to rats and humans, respectively, to assess changes in tooth movement. Aside from pharmacologic agents, Davidovitch et al[65] studied the effect of electric currents on bone remodeling and tooth movement in cats, while Stark and Sinclair[66] looked at the effect of pulsed electromagnetic field on bone remodeling in guinea pigs. It was thought that application of an electric current and force would move teeth more quickly than force alone. More recently, laser therapy has been studied to evaluate the effects on tooth movement.[67] [68,69]

Rajasekaran et al[70] performed a split-mouth study in human subjects comparing canine retraction after corticotomies on one side and 100 mcg of prostaglandin E1 injected on the contralateral side. The average rate of space closure for the prostaglandin

E1 injections was 0.36mm/week with a standard deviation of 0.05mm/week while the rate of space closure for the corticotomy side was 0.40mm/week with a standard deviation of 0.04mm/week. The difference was statistically significant (P=0.003).

Although some of these studies show accelerated tooth movements, none of these approaches except prostaglandin E1, relaxin and laser therapy have been applied to humans clinically due to the potential pain, inflammation, metabolic effects and tissue damage. Laser therapy and relaxin have shown inconsistent results with most studies concluding that tooth movement is not increased in humans. With the local injections of prostaglandin E1, administrations had to be performed weekly or up to four times a week to get the desired acceleration in tooth movement. This could be seen as too much trouble for the provider. Moreover, multiple injections would need to be given at numerous sites and this process had to be repeated several times. Due to these issues, corticotomies have become the most commonly used means to accelerate tooth movement and decrease treatment time to a significant degree.

Corticotomies and the RAP

Another way to decrease bone mineral density and increase bone modeling is through surgical insult to the bone. Decortication to facilitate orthodontics began with Henrich Kole in the 1960's[71] but it was Frost who first described the rapid acceleratory phenomenon, or the RAP, in 1983.[16] He found that noxious stimuli, including but not limited to crushing injuries, fractures, and bone operations to the bone initiate the RAP, which is simply an acceleration of normal bone healing. The RAP is a biological process

that causes a temporary increase of soft and hard tissue remodeling with a rise in cellular activity and bone remodeling at the site of injury through the recruitment of osteoblasts and osteoclasts.[16] It involves an increase in perfusion, an increase in bone turnover, and a decrease in bone mineral density.

In order to understand the RAP, normal bone healing has to be understood. This includes three phases: the inflammatory phase, reparative phase and remodeling phase.[16,72-74] First is the inflammatory or reactive phase, which begins with the constriction of blood vessels to alleviate the bleeding, followed by the formation of a blood clot within a few hours. Inflammatory cells infiltrate the area, bone is resorbed and granulation tissue is formed. Cells within the granulation tissue proliferate and differentiate into fibroblasts and chondroblasts. The first phase lasts seven to 14 days. The second, the reparative phase, is when osteoid is secreted by osteoblasts, forming immature lamellar bone as the tissue is mineralized. The first two phases are typically completed within 12 weeks. Finally, the last phase involves bone modeling and remodeling into functionally competent mature lamellar bone. This last phase can take from months to years. The timing of these phases depends on the extent of the injury, the stability of the segments and the blood supply.[74] Corticotomies involve segments that are stable so these are expected to heal more rapidly.

The Wilcko brothers in 2001 hypothesized that corticotomies induced the RAP, a biological rather than mechanical process that aids in increasing the rate of tooth movement.[17-19] The corticotomy procedure as described by the Wilcko brothers, which is also known as periodontally accelerated osteogenic orthodontics (PAOO), selective

alveolar decortication (SAD), or surgically accelerated osteogenic orthodontics (SAOO), involves laying a full-thickness mucoperiosteal flap and creating perforations into the cortical bone surrounding the teeth to be moved.[20,21] The alveolar response to these bony perforations depends on the proximity to the injury and time.[75] By taking advantage of the RAP effect, it has been well documented that tooth movement can be accelerated.[17-19,30,31,76-82] The majority of the corticotomy literature focuses on mesial-distal tooth movement, which occurs within the alveolar trough, and is mostly through a more vascular and less dense lamellar bone.

Cho et al[77] performed a split-mouth study in beagle dogs. Cortical activation on the buccal and lingual was performed on the experimental side, which included 12 perforations into the cortex while taking care not to perforate completely through the cortical plate. The contralateral side served as an untreated control. A mesializing force on all third premolars was placed with a 150-gram nickel titanium coil spring. It was found that approximately 440% more tooth movement occurred on the experimental side of the maxilla, and approximately 260% more on the experimental side of the mandible when compared to the control side. Furthermore, the authors noted that maximum tooth movement occurred two weeks after the corticotomy procedure.

Iino et al[76] similarly found faster tooth movement with corticotomies in beagle dogs. Their study performed corticotomy cuts 1mm wide, at a depth carefully adjusted to reach the bone marrow by confirming bleeding through the cut lines. 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 rate of tooth movement.

Sanjideh et al[78] performed a split-mouth experimental study in foxhounds to determine whether corticotomy procedures increase tooth movement and to evaluate the effects of a second corticotomy procedure performed four weeks after the first. They used a bur to make buccal and lingual cortical cuts approximately 1-2mm deep. They found approximately twice as much total tooth movement in the mandible on the experimental side than on the control side. The second corticotomy procedure maintained the enhanced velocities for a longer duration but the differences in tooth movement were small.

Sebaoun et al[75] investigated the alveolar response to corticotomy as a function of time and proximity to the surgical injury in a split-mouth rat model. They performed buccal and lingual corticotomies around maxillary first molars in rats using ½-round bur. Decortication injuries consisted of 10 bur marks: five on the lingual and five on the buccal. At three weeks, they found that selective alveolar decortication significantly increased turnover of the alveolar spongiosa and that the impact of the injury was localized to the area immediately adjacent to the decortication injury. Tartrate-resistant acid phosphatase-positive (TRAP) staining was used to quantify the number of osteoclasts and histomorphometric analysis was used to study the alveolar spongiosa. The results found three times the amount of osteoclasts and a two-fold decrease in calcified spongiosa bone surface in the surgical group as compared to the controls. However, this significant increase in tissue turnover dissipated to a steady state by the eleventh week.

Bogoch et al[83] described the effect of an osteotomy on bone volume and bone turnover in the distal medial femoral condyle of rabbits. In a split-body study, simple, undisplaced osteotomies were performed on the condyles and the rabbits were left to heal without fixation for four weeks. They found that the osteotomy procedure stimulated new bone formation not only in the osteotomy gap but also in the tissue adjacent to the defect. There was a five-fold increase in new bone formation without a change in bone volume. This increase in bone remodeling is similar to Frost's RAP description in which there is an increase in all of the normal tissue processes, including bone modeling and remodeling, after surgical trauma. Bone turnover at remote sites were not affected, further showing that the acceleration of bone turnover is localized to the area closest to the injury.

Mostafa et al[79] compared the corticotomy-facilitated (CF) orthodontics to traditional orthodontics. They measured tooth movements and histological changes in dogs. Second premolars were extracted on all of the dogs and mini screw implants were placed in the maxilla to retract the first premolars into the extraction space with NiTi closing coils. Corticotomies were performed on the right side only using a #2 long shank round bur extended barely into the spongy bone mesial, distal and apical to the tooth being moved. Results showed that the CF technique doubled the rate of orthodontic tooth movement. Histologic findings showed more active and extensive bone remodeling in the CF group on both compressive and tension sides suggesting that the acceleration of tooth movement associated with corticotomy is due to increased bone turnover. It is

important to note that all of these animal studies performed corticotomies with flaps but used different depths of perforations in their surgical protocol.

There are limited prospective human studies that also show the relationship between corticotomies and faster tooth movement. Fischer et al[81] performed a randomized split-mouth study using six consecutively treated patients with bilaterally partially impacted canines. A series of circular holes were made with a 1½ mm round bur mesial and distal to the canines to a depth not specified by the researchers. Compared to the side using traditional canine exposure techniques, corticotomies mesial and distal to the canine decreased treatment time 28-33% (11.5 months compared to 16.6 months). The periodontal conditions of both sides were not significantly different post treatment.

Lee et al[80] performed a case-control study using adult Korean females with bimaxillary dentoalveolar protrusion. They compared 29 patients in the conventional orthodontics group with patients who received corticotomies plus orthodontics with skeletal anchorage maxilla. The corticotomy-assisted group completed treatment in 19 months (±6 months) compared to 27 months (±7 months) for the control group. The problem with this study was that the groups were not equivalent at the start of the study and randomization of treatment assignment was unclear.

A study by Aboul-Ela et al[82] evaluated miniscrew supported canine retraction with and without corticotomies in thirteen adults with Class II, Division I malocclusion who required first premolar extraction. Using a #2 round bur, corticotomy perforations were made on the buccal bone, extending from the lateral incisor to the first premolar

area. The depth of the holes approximated the width of the buccal cortical bone. The average daily rate of canine retraction was significantly (two times) higher on the corticotomy side than on the control side during the first 2 months after the corticotomy surgery. Differences in tooth movement decreased to 1.6 times higher during the third month and 1.06 times higher during the fourth month. This study shows that corticotomies increased rate of tooth movement during the first three months only.

Greater Surgical Insult

There is a limited amount of evidence in the literature showing that the amount of surgical insult can affect the rate of orthodontic tooth movement. The greater the surgical insult, the greater the tooth movement accelerates. However, the duration of the RAP is not extended.

Mostafa et al[79] combined extractions with corticotomies to enhance the surgical effect and compared this to extractions alone in dogs to show that the increase in surgical insult increased the rate of tooth movement by twice as much in the first week. They were the first to demonstrate that performing corticotomies along with extractions produced greater tooth movements than extractions alone. Comparably, Sanjideh et al[78] performed a split-mouth study with a similar design (extractions alone versus extractions plus corticotomies) and found approximately twice as much tooth movement on the experimental side compared to the control.

Other studies such as Cho et al[77] and Iino et al[76] performed extractions and allowed a healing period before performing the corticotomies to assess the RAP effect of

corticotomies without the added surgical insult of extractions. While these studies also found an increase in the velocity of tooth movement compared to the controls, the actual amount of tooth movement in millimeters was less in both groups when compared with the studies that combined extractions and corticotomies. By comparing the various studies, the evidence indirectly supports the fact that an increase in surgical insult to the bone increases the RAP.

There has only been one study that has been designed to evaluate the effect of greater and lesser surgical insult on bone. Cohen et al[30] used a split-mouth design in foxhounds to compare the effects of two surgical techniques on tooth movement. The surgical insult on the first group (RAP group) involved extraction of the maxillary first premolars, removal of the inter-septal bone within 1 mm of the second premolars and creation of buccal and lingual vertical grooves within the extraction socket. In the second group (RAP+ group), the maxillary first premolars were also extracted and the inter-septal bone removed 1 mm from the second premolars. A full thickness mucoperiosteal flap was laid and the buccal plate between the second premolar and canine was removed. Osteotomies were then performed extending to, but not through, the lingual cortex 1 mm distal and apical to the second premolar. This study found that the increased surgical trauma, and therefore, an increase in RAP, increased the rate and ultimately the amount of tooth movement.

McBride et al[31] evaluated the same dogs to quantify the amount and maturity of the dentoalveolar bone around the teeth that have been moved on the RAP and the RAP+ sides. They found that there was significantly less bone on the RAP+ side; it was less

dense and less mature than the bone on the RAP side. Histologic evaluations indicated an increased number of osteoclasts and greater bone surface areas on the RAP+ side than on the RAP side, but there were no differences in bone volume. These two studies combined demonstrated that greater surgical insults produced a larger RAP response, characterized by less dense and less mature bone, and these changes were made possible by increased osteoclastic activity. These changes translated to greater amounts of tooth movement. The problem with this study design is that different surgeries were done to induce the increase in trauma. Not only were corticotomies performed, but they were combined with extractions and in some cases removal of the cortical plate. It is difficult to prove with this study design that it was the increase in cortical damage that facilitated the increase in tooth movement rather than the different surgeries performed.

While the studies increasing surgical damage did find a greater acceleration of tooth movement, none of the surgical procedures they described can be applied clinically in non-extraction cases because they increased the surgical insult by extraction of adjacent teeth and/or by causing excessive damage to the bone by removing the entire cortical plate. Removing the cortical plate, while done in some case reports, is not considered standard of care in most orthodontic cases, particularly in non-extraction cases. A more realistic approach to increasing surgical insult is to increase the number of cortical perforations that are made between the teeth. However, no study to-date has quantified the effects of increasing cortical damage on the cortical bone adjacent to the surgical sites in a controlled manner that a split-body design can provide.

The purpose of the current study is to evaluate the bone turnover and bone density of adjacent cortical bone when cortical damage is limited to one osteoperforation compared to four osteoperforations of the same size. The study will be performed in a split-body design on the tibias of skeletally mature New Zealand white rabbits with each rabbit serving as its own control. This study design will also allow for the examination of how much the RAP changes with a quantifiable change in the volume of cortical damage since each osteoperforation has the same dimensions. The hypothesis is that the bone adjacent to the site of increased cortical damage will show greater osteopenia and decreased bone density, which will ultimately allow for faster tooth movement.

CHAPTER II

THE EFFECT OF INCREASED CORTICAL DAMAGE ON BONE DENSITY AND BONE TURNOVER IN MATURE RABBITS: A RANDOMIZED SPLIT-BODY STUDY

INTRODUCTION

According to the American Association of Orthodontists, the average length of comprehensive orthodontic treatment is 22 months[1], but treatment times can last up to 36 months for patients with severe orthodontic problems.[1-5] For nonextraction cases, treatment durations range from 21-27 months, while extraction treatment ranges from 25-35 months.[1,3-7,84] Extended treatment time has been associated with an increased rate of root resorption,[8-12] decalcification and caries,[11,13] periodontal problems[14,15] and increased costs to the orthodontist and patient. As orthodontists, it is important to consider methods to decrease treatment time in order to minimize these risks while still obtaining satisfactory results. One way to reduce treatment time is to increase the rate of tooth movement.

Currently, performing corticotomies is the best-established surgical intervention used to accelerate tooth movement by inducing the regional acceleratory phenomenon (RAP).[16] The RAP is a biological process that causes a temporary increase of soft and hard tissue remodeling with a rise in cellular activity and bone remodeling at the site of injury through the recruitment of osteoblasts and osteoclasts.[16] It involves an increase in perfusion, an increase in bone turnover, and a decrease in bone mineral density. The

Wilcko brothers in 2001 hypothesized that corticotomies induced the RAP, a biological rather than mechanical process that aids in increasing the rate of tooth movement.[17-19] The corticotomy procedure as originally described by the Wilcko brothers, which is also known as periodontally accelerated osteogenic orthodontics (PAOO), selective alveolar decortication (SAD), or surgically accelerated osteogenic orthodontics (SAOO), involves laying full-thickness mucoperiosteal flaps labially and lingually and creating perforations into the cortical bone surrounding the teeth to be moved.[20,21] Particulate bone grafting material is then placed over the alveolar bone and the flaps are sutured back together. There is considerable variation in the surgical techniques used depending on the surgeon's preference.

More recently, there has been a trend toward less invasive methods to create bone damage and induce the RAP. Surgical techniques that do not require reflection of the gingival tissues have been popularized due to the decrease in trauma to the patient and the potential to preserve periodontal support. Dibart was the first to introduce flapless alveolar decortication using a piezosurgery unit and termed the procedure "piezocision."[22] Subsequent studies have evaluated the efficacy of flapless corticotomies on tooth movement using various instruments such as surgical blades[23-25], carbide burs[25,26], bone awls[27] and the PROPEL device[28,29]. The PROPEL device creates micro-osteoperforations (MOPs) by threading a 1.4 mm diameter stainless steel mini-screw implant through the gingiva and into the bone. The assumption that many of these studies make is that any amount of injury to the bone can induce the RAP and accelerate

tooth movement. However, there have been limited studies evaluating the effect of greater and lesser damage to the bone.

There has only been one study that has been designed to evaluate the effect of greater and lesser surgical insult on bone. Cohen et al[30] used a split-mouth design in foxhounds to compare the effects of two surgical techniques on tooth movement. The surgical insult on the first group (RAP group) involved extraction of the maxillary first premolars, removal of the inter-septal bone within 1 mm of the second premolars and creation of buccal and lingual vertical grooves within the extraction socket. In the second group (RAP+ group), the maxillary first premolars were also extracted and the inter-septal bone removed 1 mm from the second premolars. A full thickness mucoperiosteal flap was laid and the buccal plate between the second premolar and canine was removed. Osteotomies were then performed extending to, but not through, the lingual cortex 1 mm distal and apical to the second premolar. This study found that the increased surgical trauma, and therefore, an increase in RAP, increased the rate and ultimately the amount of tooth movement. A companion study found that there was significantly less bone on the RAP+ side compared to the controls and it was also less dense and less mature.[31] Histologic evaluations indicated an increased number of osteoclasts and greater bone surface areas on the RAP+ side than on the RAP side. These two studies combined demonstrated that greater surgical insults produced a larger RAP response, characterized by less dense and less mature bone, and these changes were made possible by increased osteoclastic activity. These changes translated to greater amounts of tooth movement. The problem with this study design was that different

surgeries were done to induce the increase in trauma. Not only were corticotomies performed, but they were combined with extractions and in some cases removal of the cortical plate. It is difficult to prove with this study design that it was the increase in cortical damage that facilitated the increase in tooth movement rather than the different surgeries performed.

No study, to date, has quantified the effects of increasing cortical damage on the cortical bone adjacent to the surgical sites in a controlled manner that a split-body design can provide. The purpose of the present study was to investigate the biological changes in bone density and bone modeling of the cortical bone when increasing the amount of cortical damage.

MATERIALS AND METHODS

Ten skeletally mature New Zealand white female rabbits, approximately 6 months of age and weighing between 4-6 kg, were acquired for the study from an approved breeder. Housing, care, and experimental protocol were approved by the Institutional Animal Care and Use Committee at Texas A&M University College of Dentistry (IACUC #2015-0243-BCD). After acquisition, the rabbits were acclimated for seven days. During this time, their vital signs and weights were monitored every three days. A prospective split-body design was used to compare the right and left tibias. The tibias were used because they are larger than their maxilla or mandible, which makes it possible to more easily standardize and quantify osteoperforations (OPs). One operator performed all of the surgeries. Two pilot rabbits were acquired prior to the study to

establish the surgical procedures and eliminate any unforeseen complications in the experimental design.

Surgical Protocol

Using a random number table generator, each rabbit tibia was randomly allocated to either one OP (control) or four OPs (experimental). On the day of the surgery, the first four animals were placed under general anesthesia of ketamine: xylazine 50 mg/kg:10 mg/kg given intramuscularly. Because two of the rabbits were lost due to cardiac problems, rabbits 5 through 10 were anesthetized using inhaled isoflurane. The rabbits were placed in an induction chamber and 1-1.5% isofluorane in oxygen was administered at a rate of 1 L/min. Once the rabbits showed signs of relaxation, the isoflurane was maintained via a gas mask placed tightly over each rabbit's snout. At this time, the anterior surfaces of both tibias were shaved and local anesthesia of 2% lidocaine with 1:100k epinephrine was administered intramuscularly at each surgical site. Full thickness periosteal flaps were laid on the anterior surface of each tibia to peel back the soft tissue layer in order to expose the underlying bone. In order to standardize the location of the osteoperforations, 3 mm long titanium mini screw implants (MSIs) with a diameter of 1.6 mm, were placed 30 mm apart on the proximal 1/3 of each tibia. Pilot holes approximately 1 mm deep were created with a 1 mm diameter pilot hole bur on a slow speed hand piece in order to avoid bone fracture during MSI placement. Using a hand driver, MSIs were placed until all of the threads were in the bone. The OPs were drilled 15 mm away from each MSI. On one randomly chosen side, one OP was made

with a 1.5 mm round bur attached to a high-speed hand piece through the cortical bone for the control group (Figure 1A). Using the same round bur, four OPs were made through cortical bone on the experimental side (Figure 1B). Adequate air and irrigation were used to maintain a clear surgical field and to prevent necrosis of the bone. The tissue was reapproximated and sutured closed with simple interrupted sutures using 3-0 Vicryl resorbable sutures. Two weeks after the surgeries, the rabbits were sacrificed via exsanguination under surgical plane anesthesia using heparinized saline. The tibias were harvested and sectioned to include the initial OPs, along with up to 3 mm of cortical bone adjacent to the cuts on either side. All of the soft tissue around the samples was removed. The harvested samples were stored in 4% paraformaldehyde (PFA) for 1 week prior to analysis.

Data Collection and Analysis

MicroCT- Bone Density

Each of the samples were placed in a vial, immersed in 4% PFA, and scanned with Bruker's Skyscan 1173 with the following settings: 60 kV energy level, 167 uA current, 0.5 mm aluminum filter, 0.7° rotation step, 4 frame averaging, medium camera resolution setting (2000 pixel field width), 800 ms exposure and 10 micron size. Scan time was approximately 20 minutes for each specimen.

The cortical bone was divided into two regions of interest (ROIs) (Figure 1A and 1B). Each ROI was 7 mm long, 1 mm wide and included the entire thickness of the cortical bone, which was 2 mm on average. The same ROIs were defined for analysis for

both the control and experimental sides. ROI 1 started 0.01 mm from the OPs and extended 1.01 mm away from the OPs. ROI 2 started 1.01 mm from the OPs and extended out up to 2.01 mm. Within each region, 100 lateral 2D slices of the bone, 0.01 mm wide and including the entire thickness of the cortical bone, were analyzed. Bone mineral densities of the cortical bone for each slice were calculated in g/cm^3.

Microhardness

The hardness of the bone samples was measured using a FM-1e Digital Microhardness Tester (Future-Tech Corporation) by one blinded investigator. To prepare the samples for testing, the tibial sections were cut cross-sectionally to include only the anterior cortical bone where the OPs were made. Then, these were cut in halves perpendicular to the OPs. Microhardness analyses were performed on the cortical bone on one half, while the other half was used for histology. The samples were then embedded in orthodontic resin (Dentsply) and polished to achieve a smooth surface for testing. The Economet 3 Variable Speed Grinder-Polisher was used with 5 different grits of sandpaper discs (200, 400, 600, 800, and 1200 grit). Each sample was polished with each grit for 1 minute starting with the more coarse 200 grit and finishing with the finest 1200 grit. The polished surfaces were then tested for microhardness at 150 μm intervals starting 150 μm away from the OPs and extending out up to 3,300 μm away from the OPs. A Vickers diamond probe was used at 100 gf with a dwell time of 15 seconds to create a microindent in the bone (Figure 2). Prior to each use of the machine, calibration of the FM-1e was performed using a metal of known hardness provided by the

manufacturer. The resulting images were analyzed with the software ImageJ (U.S. National Institutes of Health, Bethsda, Maryland, USA) in order to measure the point-to-point distance on each microindent image. This distance was then used to calculate the Vickers Hardness (HV) of the samples using the formula HV=1.8544*F/d2, where F represents the force in kg and d represents the length of the diagonal of the indentation in mm (Figure 2).

Histology- H&E and TRAP

The samples obtained for H&E (hematoxylin and eosin) and TRAP (tartrate-resistant acid phosphatase) staining were first fixed in 4% PFA, then decalcified in 0.5 M EDTA (ethylenediaminetetraacetic acid, tetrasodium salt) using the Pelco Biowave (Redding, CA). Radiographs were used to monitor the decalcification process. After the tissues had fully decalcified, the specimens were dehydrated through a graded series of alcohols, cleared with xylene, infiltrated with paraffin using the Miles Scientific VP, model 2000 automatic tissue processor, and embedded in paraffin blocks. The blocks were hardened on a cold plate, sectioned using a microtome in a sagittal direction to a thickness of 6 μm, mounted on coated glass slides, and stained with either H&E or TRAP stain. These specimens were visualized under a Zeiss Axioplan microscope (Carl Zeiss Microimaging, Germany) and photographed using SPOT 5.0 software (SPOT Imaging Solutions, Sterling Heights, MI).

To analyze the TRAP slides, the cortical bone of each specimen was divided into three sections. Section 1 started at the edge of the OPs and extended 0.50 mm away from

the OPs. Section 2 started at 0.50 mm away and extended 1.0 mm, and Section 3 started at 1.0 mm up to 1.5 mm away from the OPs (Figure 3A and 3B). The blood vessels with surrounding osteons undergoing osteoclastic remodeling were counted as well as the total number of blood vessels to create a ratio to determine the osteoclastic activity for each section (Figure 3C). The slides were visualized and the counts were performed under 10x magnification by a single blinded investigator.

Statistical Analysis and Determination of Significance

All statistical analyses were performed using SPSS® 22.0 software (SPSS Inc.: Chicago, IL). Data for the TRAP was described using means and standard deviations. The experimental and control specimens were compared using 1-tailed Student's t-tests with a significance level of $p<0.05$. Multilevel statistical models were used to statistically determine the shape of the curve describing bone density, microhardness and the differences between the experimental and control sides. The multilevel models were developed using the MLwiN software (Version 2.01, Center for Multilevel Modeling, Institute of Education, London, UK).

RESULTS

Healing of the surgical sites progressed normally with minimal to no swelling, and no infection in all of the animals. Rabbit 8 broke a tibia prior to the two-week sacrifice and was excluded from the study.

MicroCT

The three-dimensional reconstructions showed distinct holes where the surgeries were performed, as well as some bone formation in the holes after 2 weeks of healing (Figure 4). In the control samples, a single osteoperforation was evident from the coronal view and in the experimental samples, four osteoperforations could be visualized (Figure 4C and 4D). In some of the control and experimental samples, there was a significant amount of bone formation within the bone marrow space adjacent to the sites of injury (Figure 4A and 4B).

The control bone slightly increased in density as the distance from the OPs increased and this change was statistically significant ($p<.001$) ((Figure 5 and 6). The experimental bone also increased in density as the distance from the OPs increased, but the change was not statistically significant. The experimental bone was significantly ($p<.001$) less dense than the control bone for both zones and at all distances away from the OPs.

Microhardness

The microhardness of the control bone gradually increased as the distance from the OPs increased (Figure 7). The experimental bone showed significantly greater increases in microhardness as the distance from the OPs increased. The control bone was significantly ($p<.001$) harder than the experimental bone at all distances.

Histology

H&E Staining

The H&E sections of cortical bone showed greater amounts of woven bone within the injury site in the control specimen than in the experimental specimen after 2 weeks of healing (Figure 8A and 8B). There was a greater amount of unmineralized fibrous tissue within the injury site in the experimental group compared to the control group (Figure 8A and 8B). In both experimental and control specimens, there was added tissue on the endosteal and periosteal surfaces. (Figures 9A and 9B). Most of the added tissue was found on the periosteal surface, and only half of the specimens had added tissue on the endosteal surface. In the control bone, the added tissue was primarily woven bone (Figure 9A). In the experimental bone, the added tissue was mainly unmineralized fibrous tissue with little mineralized woven bone (Figure 9B). In both specimens, there were large numbers of osteoclasts lining areas of remodeling and several osteoblast-lined surfaces (Figures 10A and 10B). Additionally, there were areas of acellularity directly adjacent to the injury in almost all of the control and experimental samples (Figures 11A and 11B). These acellular areas had empty lacunae with no osteocytes. These acellular areas extended up to 0.875 mm away from the injury.

TRAP Staining

The histologic sections treated with TRAP staining clearly displayed where there were areas of osteoclastic activity (Figures 12A and 12B). Within the site of injury, there was extensive TRAP activity in both the control and experimental groups. Based on the

ratio of blood vessels with surrounding osteons undergoing osteoclastic remodeling over the total amount of blood vessels, the experimental bone had significantly ($p<.05$) greater osteoclastic activity than the control bone in Sections 1 and 2 (Figure 13). Osteoclastic activity was also greater on the experimental side in Section 3, but this was not statistically significant.

DISCUSSION

It has been well established that the density of bone adjacent to trauma sites decreases. Dual-energy x-ray absorptiometry has shown that fractured human tibias evaluated up to five months after injury are less dense than the unfractured tibias, both within the fracture site and in the region proximal to the fracture.[85] Bone surrounding micro-osteoperforations (MOPs) created with the PROPEL appliance also undergoes a significant decrease in cortical and trabecular bone density around each MOP during the first 2-4 weeks.[28] Similarly, bone subject to piezocision shows greater demineralization than control bone for up to 28 days after the injury.[86]

The decrease in bone density associated with trauma is due to the regional acceleratory phenomenon (RAP) as first described the by Frost in 1983.[16] Noxious stimuli to bone, such as crushing injuries, fractures, and bone operations initiate the RAP. The RAP is a biological process that causes a temporary increase of soft and hard tissue remodeling with a rise in cellular activity and bone remodeling at the site of injury through the recruitment of osteoblasts and osteoclasts. There is an increase in bone turnover as well as a decrease in bone mineral density. The Wilcko brothers were the

first to hypothesize that corticotomies induce the RAP.[17-19] Several experimental studies have shown that decortication causes demineralization.[17-19,75,87,88]

The greater the surgical insult, the greater the decrease of cortical bone density. In the present study, the bone mineral density was significantly less in the specimens with four OPs than in the specimens with only a single OP. While injury compared to no injury has been widely studied, only one other experimental study has compared the osseous effects of greater and lesser injury.[30,31] On one side, the maxillary first premolars were extracted, the inter-septal bone was removed to within 1 mm of the second premolars and buccal and lingual vertical grooves were cut within the extraction socket. The other side underwent a greater surgical insult. The maxillary first premolars were also extracted and the inter-septal bone removed to within 1 mm from the second premolars. In addition, a full thickness mucoperiosteal flap was laid, the buccal plate between the second premolar and canine was removed, and osteotomies were then performed extending to, but not through, the lingual cortex 1 mm distal and apical to the second premolar. MicroCT analyses showed significantly less bone, less dense bone and less mature bone on the side with the greater surgical insult than the bone on the RAP side.[31] Histologic evaluations indicated an increased number of osteoclasts the side with greater insult.[31] A companion study showed significantly faster tooth movements on the side with greater surgical insult.[30] Due to the variety of procedures used on the side with greater insult, it is impossible to ascribe the differences solely to the amount of surgical insult. The type of surgical insults could also have played a role. Because the present study design performed the same type of insults on both sides and varied only in the

amount, any differences that were identified must have been due to the amount of surgical insult.

Greater surgical damage also results in bone near the site of injury that is less hard. In the current study, Vickers hardness at all distances away from the cortical damage was greater in the specimens with one OP than in the specimens with four OPs. Van Gemert et al[28] reported significant decreases in the Vickers hardness of cortical bone around MOPs extending up to 0.75 mm from the injury site. Stea et al[89] found that bone near stainless steel pins inserted into sheep tibias was less hard closest to the pin than further from the pin. A study evaluating the mechanical properties of bone adjacent to osteotomy gaps found that the sites furthest from the osteotomy were harder than sites closest to the osteotomies.[90] Additionally, this study found that the greater gap between bone segments resulted in bone that was less hard compared to a smaller gap between bone segments, which supports the idea that greater injury to the bone results in a decrease in hardness. Associations between hardness and density have been previously established. In a study of human iliac bone, both microhardness and the degree of bone mineralization were significantly lower in osteoporotic patients than in the controls.[91] The same study also found significant positive correlations between the Vickers hardness and the bone mineral density.[91] A study on human cadaver bone has also reported significant positive correlations between microhardness and microradiodensity.[92] Fluoride treatment in rabbits causes an increase in bone mineralization and an increase in microhardness, indirectly showing a positive correlation between the two measurements.[93] Hardness and bone mineral density are

expected to be related because hardness is largely dependent on the mineral content of the bone, especially in the amount of calcium.[94] A study analyzing the material properties of a fracture callus of the rat tibia found that hardness was correlated with the mineral density.[95] Additionally, this study found that bone density was linearly correlated with concentrations of hydroxyapatite and calcium. Bone mineral density is a volumetric measure of the calcium hydroxyapatite and therefore any changes in calcium will affect density as well as hardness.

Bone density and hardness undergo greater reductions with greater surgical insult because bone resorption is increased. In the present study, TRAP staining showed greater osteoclastic activity around the blood vessels of the specimens with four OPs than in the specimens with one OP at all distances away from the injury. A split-mouth rat study showing greater bone demineralization with piezocision also reported an associated increase in osteoclastic activity immediately following injury.[86] A dramatic increase in TRAP activity has also been reported in the cortical and trabecular bone two weeks after MOPs were performed.[28] Other studies using beagle dogs and rats have also found that bone decortication results in a higher number of osteoclasts, further supporting the fact that injury to the bone causes an increase in bone resorption.[75,77]

There were acellular areas evident in the cortical bone extending away from the injury. In a majority of the specimens, there was an area immediately adjacent to the OPs that was devoid of osteocytes, where empty lacunae could be visualized. There was no consistent pattern regarding the extent of the acellularity, and it did not appear to be related with the amount of injury. Empty osteocyte lacunae provides histological

evidence of osteonecrosis.[96] Using a bur to drill holes in bone could generate enough heat to cause osteocyte apoptosis.[96,97] In rabbits, it has been shown that a temperature of 55°C for 30 seconds will induce irreversible osteocyte death.[98] Eriksson et al,[99] who analyzed the histology of rabbit bone after trauma, found that 47°C for 1 minute was the threshold after which thermal necrosis of the cortical bone will occur. Greater drilling speeds produce more heat, thereby increasing the chance for osteocyte apoptosis.[100,101] Using a high-speed hand piece at an average 200,000 rpm, as used in this present study, could generate heat well above the threshold for osteonecrosis.

Aside from thermal damage, drilling in bone can also cause microdamage which can also lead to osteocyte apoptosis.[102] Studies evaluating the bone strain after the placement of self-tapping miniscrew implants and the PROPEL appliance have reported strains well above the physiologic limits of the bone, i.e. strains that could cause osteocyte death.[103-105] Prior studies have also shown significant decreases in fluid shear stress associated with microcracks, leading to further osteocyte apoptosis.[106-110]

The regional acceleratory phenomenon (RAP) extends some distance from the site of trauma. In the present study, the bone mineral density was significantly less dense on the side with four OPs at all distances away from the injury up to 2.0 mm, Vickers microhardness of the cortical bone was significantly less up to 3.5 mm away from the site of trauma, and the TRAP staining displayed dramatic osteoclastic activity up to 1.5 mm away from the injury. These findings together demonstrate that the RAP effect in the cortical bone can extend up to 3.5 mm away from the surgical insult after 2 weeks of healing. Significant decreases in cortical and trabecular bone density as far as 3-4 mm

from the MOPs and significant TRAP activity at least 2.5 mm from the edge of the MOPs have been reported after two weeks of healing.[28] In rats, it has been shown that demineralization extends up to 2.5 mm away from the injury site after seven days of healing.[86] This distant RAP effect could be the result of the apoptotic osteocytes adjacent to the sites of injury communicating through their extensive canalicular network to initiate bone resorption via the recruitment of distant osteoclasts.[110-114] It has been shown that each osteocyte has an average of 89 dendritic processes, each 125 μm in length, that communicate with other osteocytes.[115] This extensive branching could extend far from the site of the original osteocyte and distant osteoclasts would be activated.

The amount of injury is also related to the rate of healing, with more rapid healing occurring in sites with less injury. H&E staining of the cortical bone sections displayed a greater amount of woven bone within the site of injury of the single OP compared to the four OPs. In the specimens with four OPs, there was a greater amount of unmineralized fibrous tissue, indicative of an earlier stage of healing. There was also added tissue on the endosteal and periosteal surfaces in both groups due to the formation of a callous. Again, there was more woven bone in the callous on the one-hole side and more fibrous tissue on the four-hole side.

Normal healing begins with the inflammatory phase, when the blood vessels are constricted to alleviate bleeding and a blood clot forms within a few hours.[72,74,116] Inflammatory cells infiltrate the area, bone is resorbed and granulation tissue is formed. Cells within the granulation tissue proliferate and differentiate into fibroblasts and chondroblasts.[116] In humans, the first phase lasts seven to 14 days. The second,

reparative phase, is when osteoblasts secrete osteoid, forming immature lamellar bone as the osteoid is mineralized. The first two phases are typically completed within 12 weeks. The last phase involves bone modeling and remodeling into functionally competent mature lamellar bone. This last phase can take from months to years.[74] In the current study, all of the specimens appear to be in the reparative phase of healing, but the one OP side consistently exhibited greater mineralized tissue, indicating faster healing than the four OP group. It is possible that since there is a greater area of damage with the four OPs compared to a single OP, it takes longer for the osteoblasts to populate the area and secrete osteoid to fill the holes, thereby extending the healing time. A study analyzing the healing rates adjacent to osteotomy sites in sheep metatarsi found that the larger the gaps between bone segments, the less complete the healing when compared to smaller gaps.[90,117] The majority of the tissue in the large osteotomy gaps was unmineralized connective tissue while greater bone and calcified cartilage was present in the smaller osteotomy gaps.

Limitations of this study include the fact that the surgeries were done on the long bone of an animal model, specifically rabbit tibias, which have very little trabecular bone. This contrasts with human jaws, which have both cortical and trabecular bone. This is important because the effects of injury on the trabecular bone also need to be known. It is also difficult to extend these findings to tooth movement. While tooth movement might be expected to accelerate with decreases in bone density and hardness and an increase in osteopenia, there may be a threshold that needs to be reached to see accelerations in tooth movement. It would be best to replicate the present study in a

larger animal in order to assess how tooth movements are affected by greater and lesser surgical insults.

CHAPTER III

CONCLUSIONS

From the present study, it can be concluded that in and around the site of injury:

1. The greater the surgical insult, the greater the decrease in cortical bone density.
2. Greater surgical damage also results in bone that is less hard.
3. Bone density and hardness are reduced with greater surgical insult because bone resorption is increased.
4. There were acellular areas evident in the cortical bone extending away from the injury.
5. The regional acceleratory phenomenon (RAP) extends some distance from the site of trauma.
6. The amount of injury is also related to the rate of healing, with more rapid healing occurring in sites with less injury.

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APPENDIX A

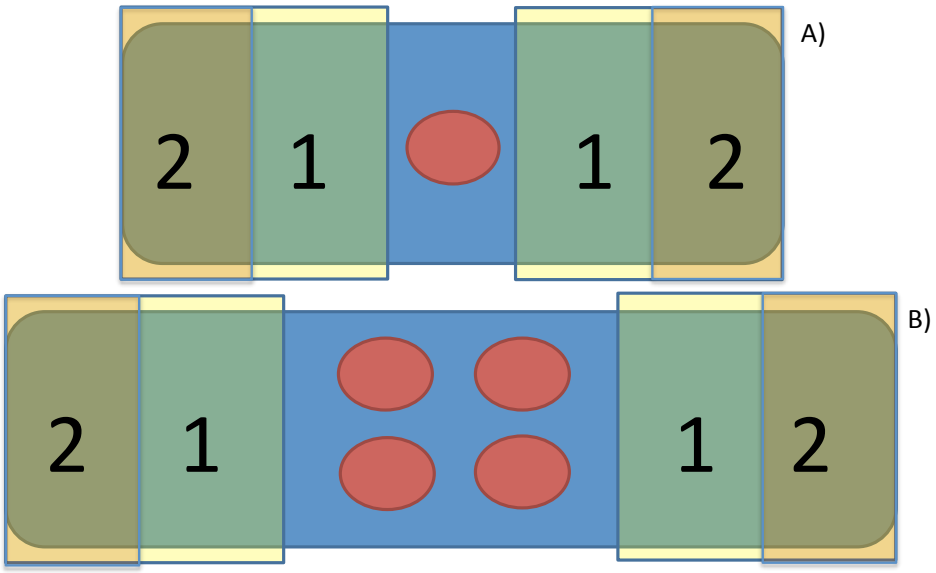

Figure 1: Regions of Interest. A) Control group, B) Experimental group both showing Region 1- closest to the osteoperforations and Region 2- furthest from the injury

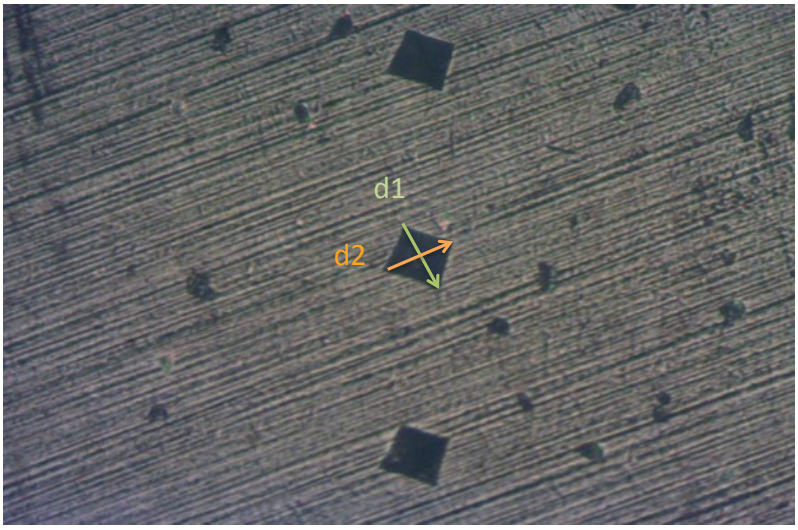

Figure 2: Microhardness Indentations and Equation. A Vickers diamond probe was used at 100 gf with a dwell time of 15 seconds to create a microindents in the bone 150 µm apart. Vickers Hardness (HV) calculated using $HV=1.8544*F/(d1+d2)/2)^2$, where F represents the force in kg and d represents the length of the diagonal of the indentation in mm

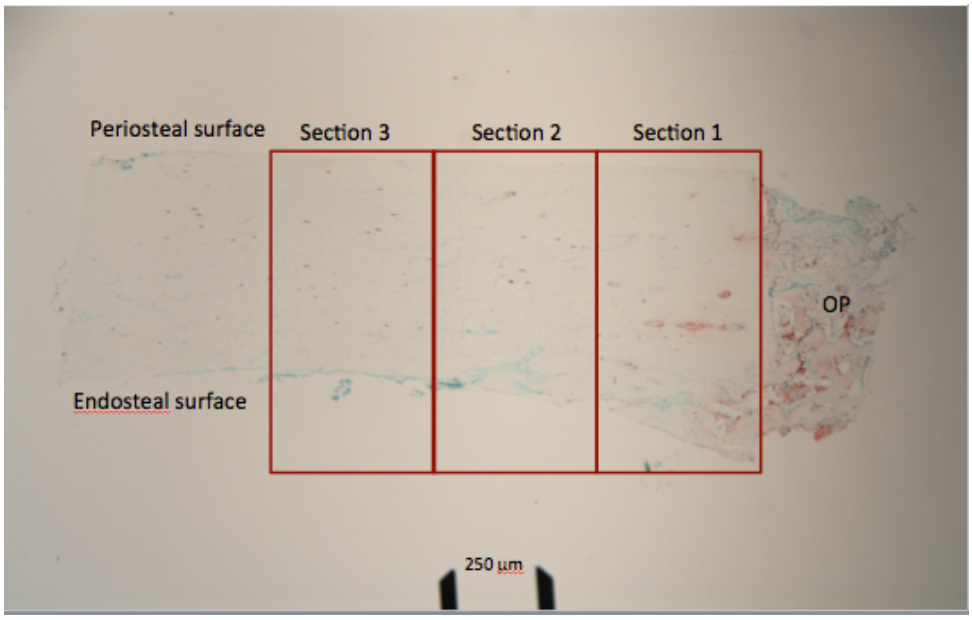

Figure 3A: TRAP Sections: Control 2.5x Magnification

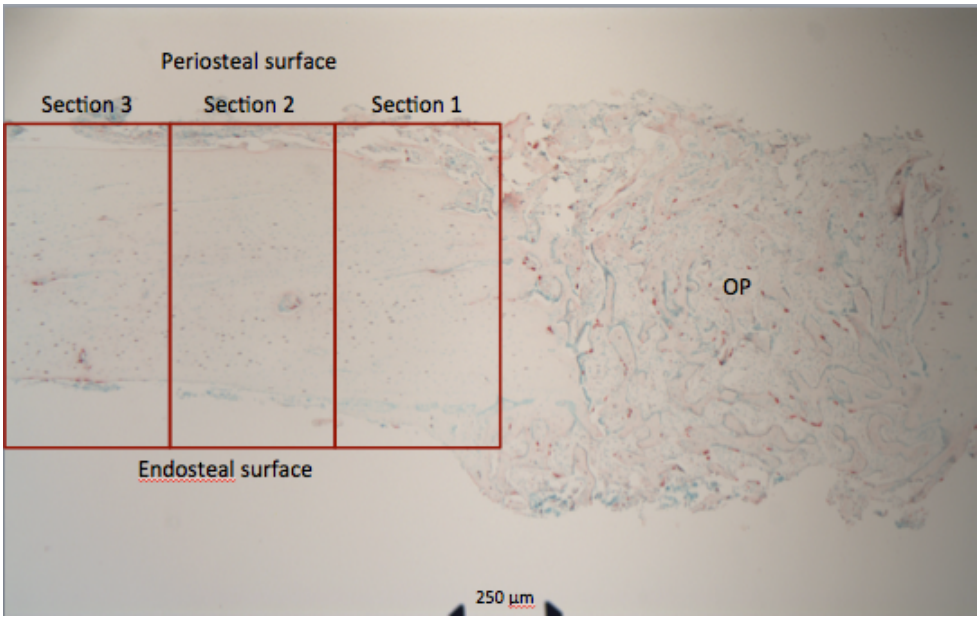

Figure 3B: TRAP Sections: Experimental 2.5x Magnification

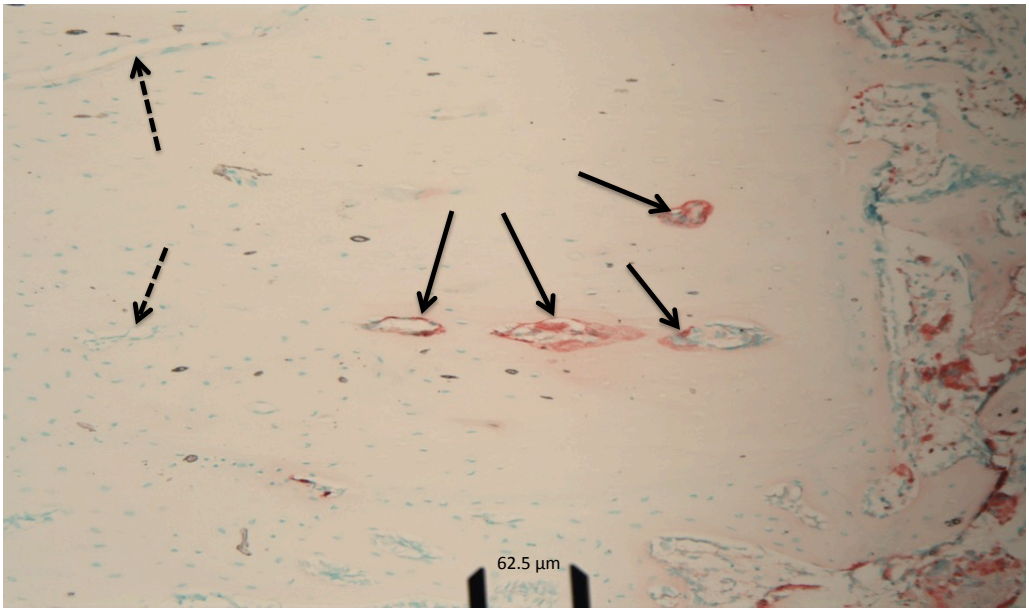

Figure 3C: TRAP Section: 10x Magnification. (→) indicates osteoclastic activity while (-->) indicates no osteoclastic activity

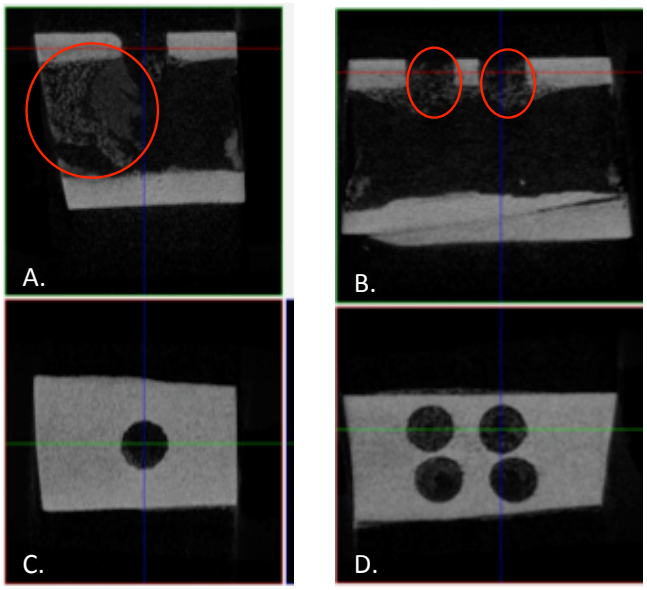

Figure 4: MicroCT 3D reconstructions. A) control sagittal view, B) experimental sagittal view, C) control coronal view, D) experimental coronal view. Circled areas show new bone formation.

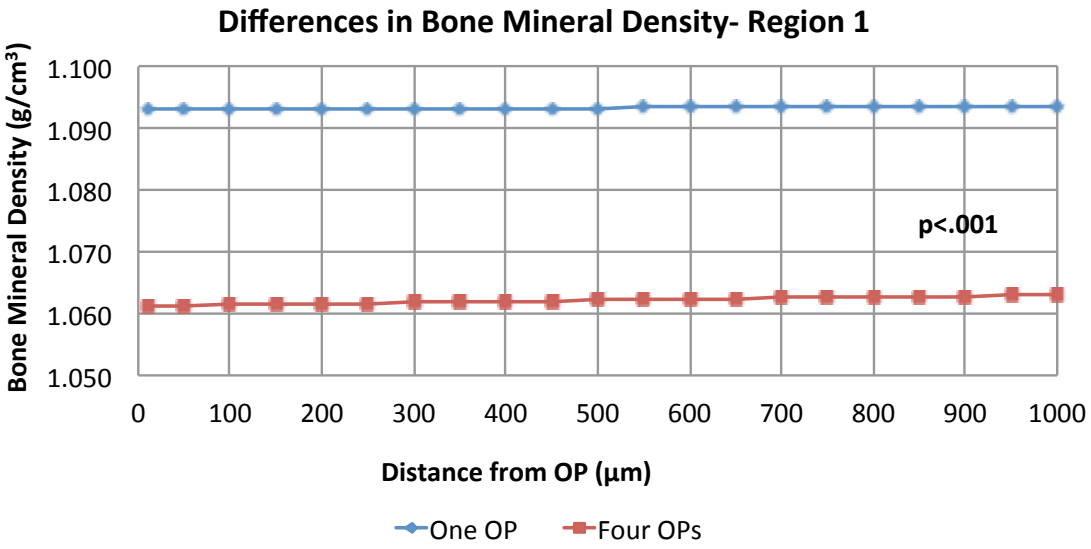

Figure 5: Differences in Bone Mineral Density- Region 1. Bone mineral density of the cortical bone in Region 1, closest to the osteoperforations, is measured in g/cm^3. Significance p<0.001 at all distances.

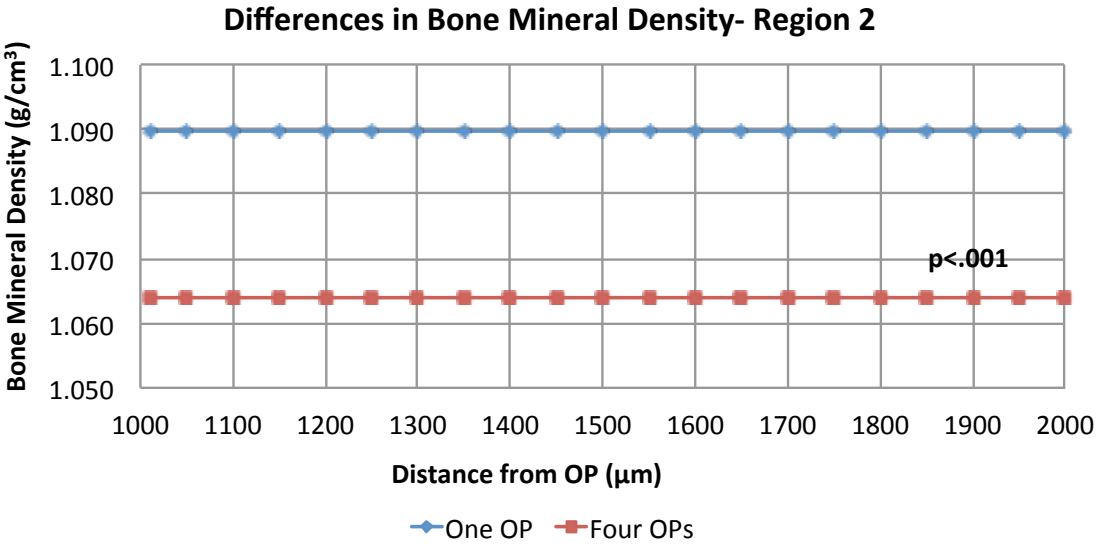

Figure 6: Differences in Bone Mineral Density- Region 2. Bone mineral density of the cortical bone in Region 2, furthest from the osteoperforations, is measured in g/cm^3. Significance p<0.001 at all distances.

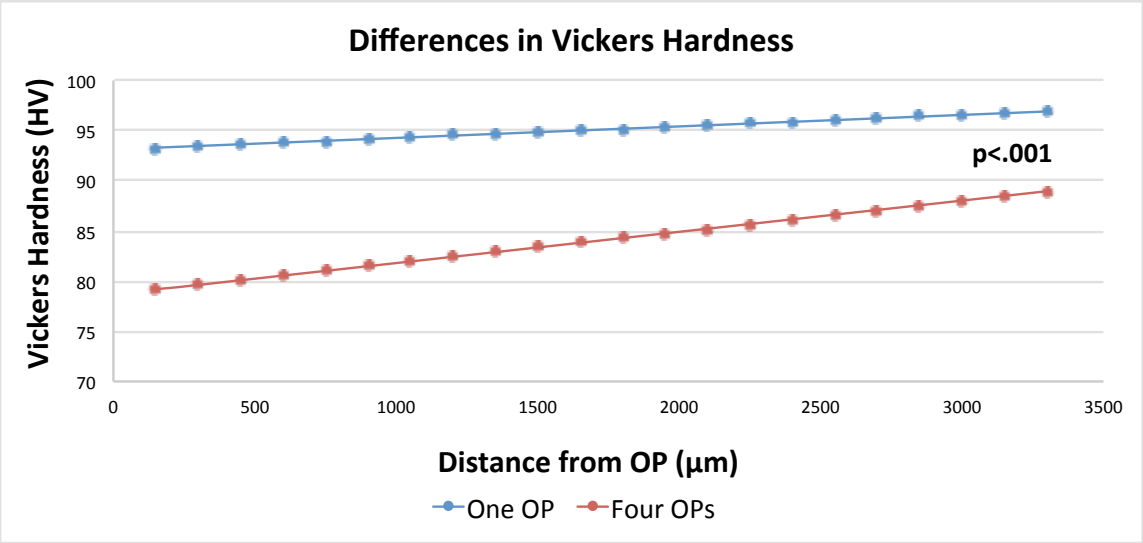

Figure 7: Differences in Vickers Hardness. Vickers hardness of the cortical bone measured in HV as the distance from the osteoperforations increases. Significance p<0.001 at all distances.

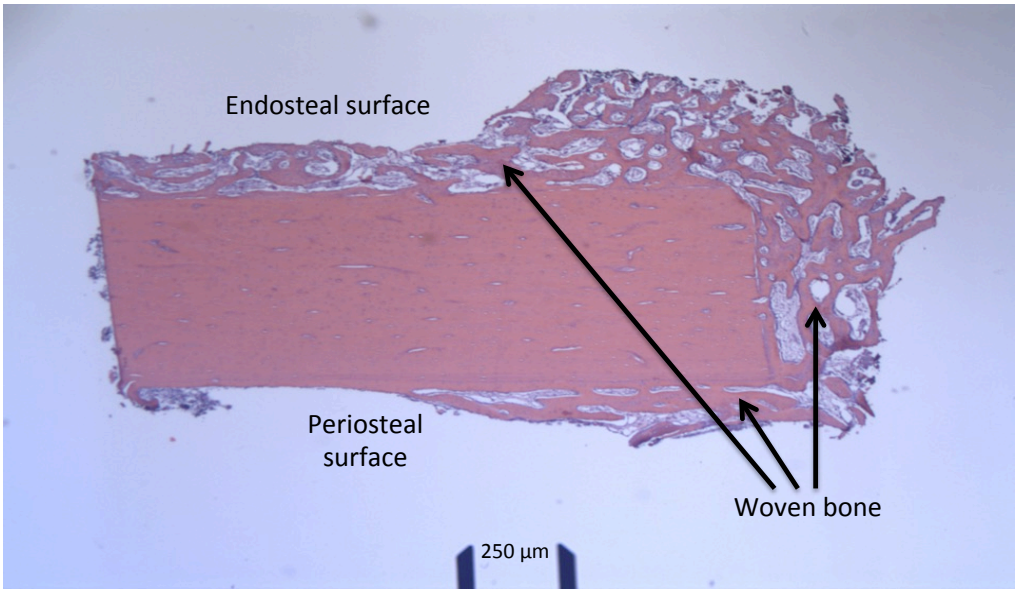

Figure 8A: H&E Section: Control 2.5x Magnification.

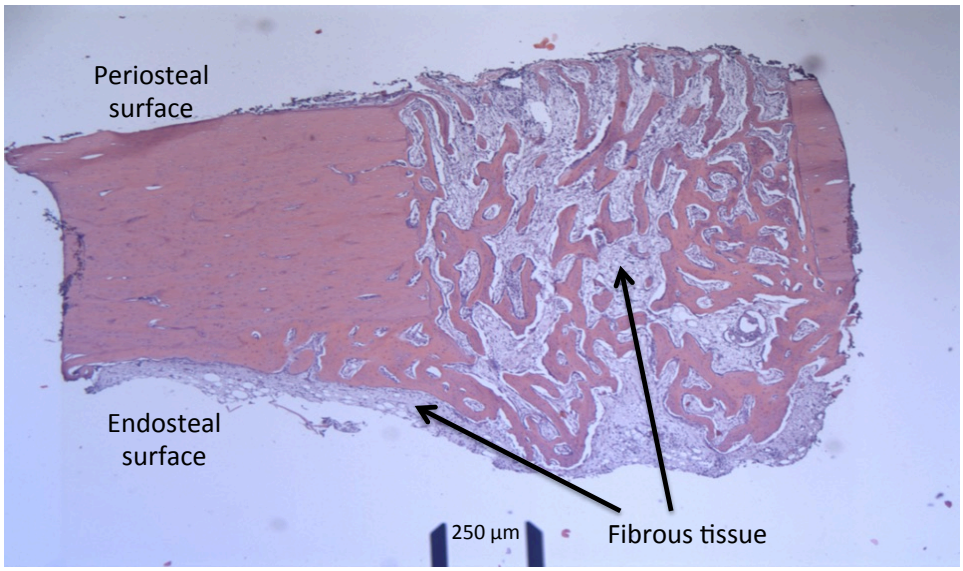

Figure 8B: H&E Section: Experimental 2.5x Magnification.

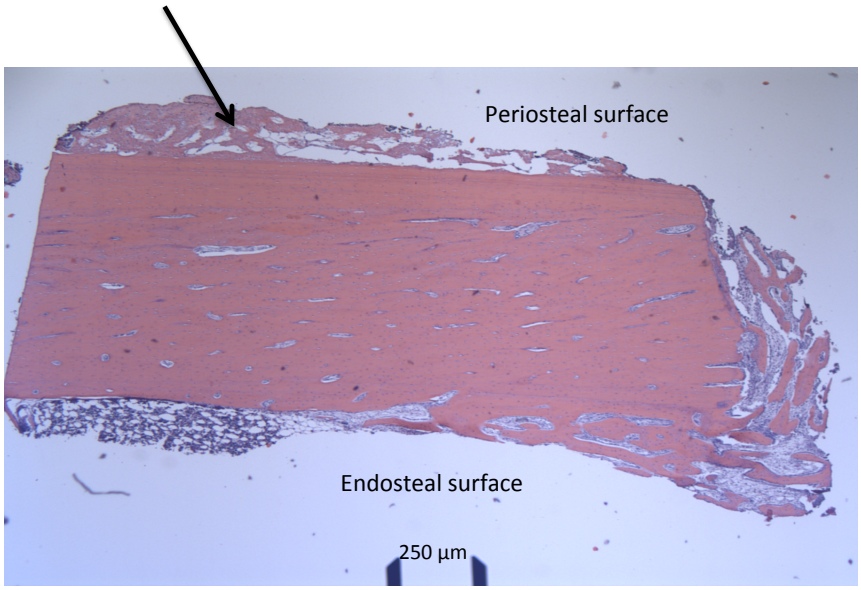

Figure 9A: H&E Section: Control, Added Tissue. (→) indicates added tissue which was primarily woven bone.

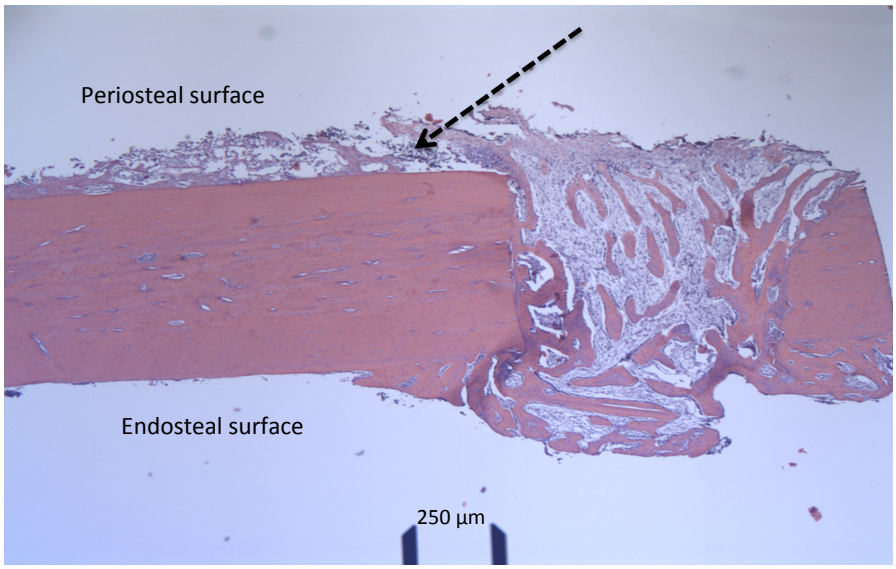

Figure 9B: H&E Section: Experimental, Added Tissue. (-->) indicates added tissue which was primarily fibrous tissue.

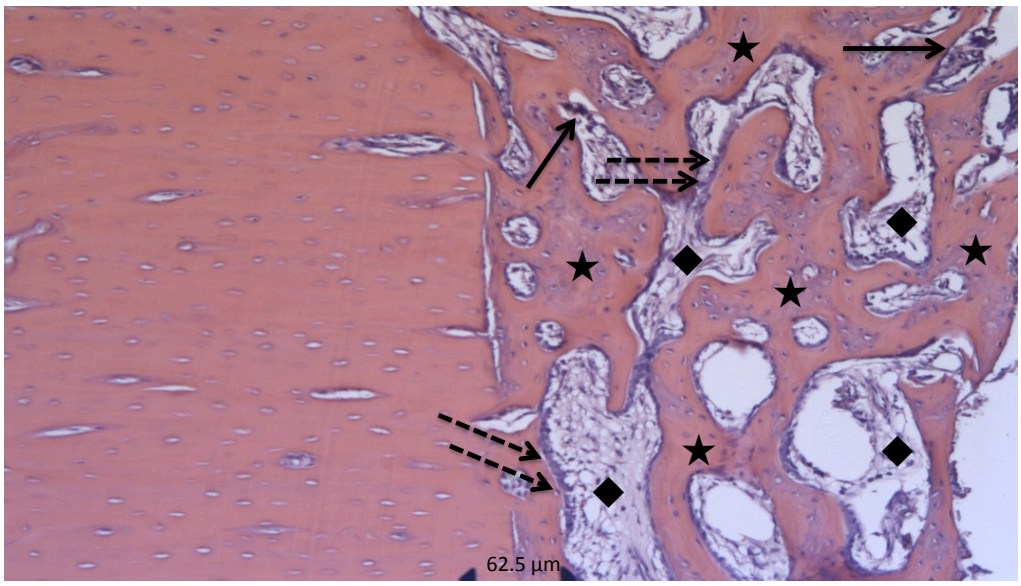

Figure 10A: H&E Section: Control 10x magnification. (★) Woven bone (◆) Fibrous tissue, (→) osteoclasts, (-->) osteoblasts

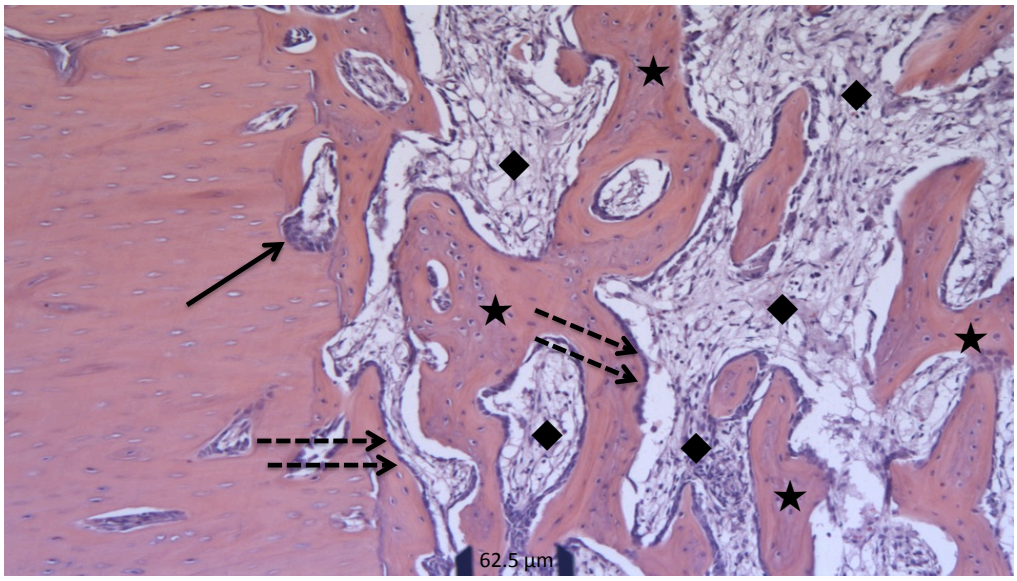

Figure 10B : H&E Section: Experimental 10x Magnification. (★) Woven bone, (◆) Fibrous tissue, (→) osteoclasts, (-->) osteoblasts.

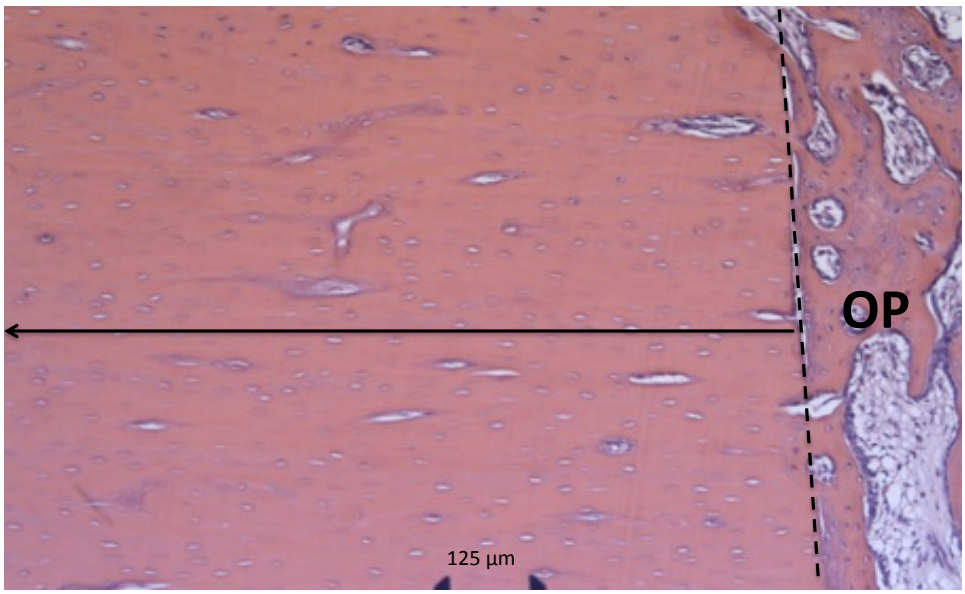

Figure 11A: H&E Section: Control, Area of Acellularity. (---) delineates site of OP, (→) indicates extent of acellularity

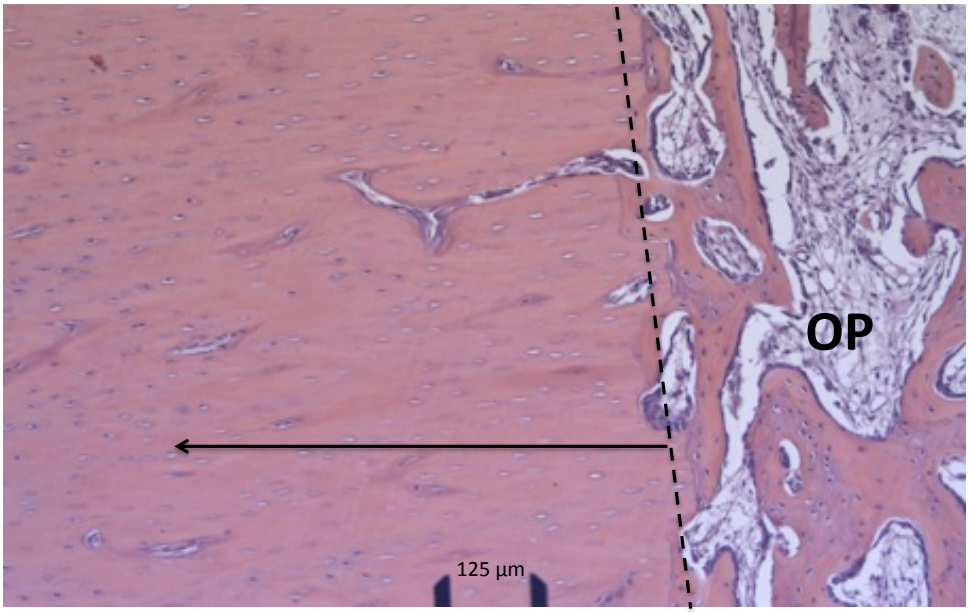

Figure 11B: H&E Section: Experimental, Area of Acellularity. (---) delineates site of OP, (→) indicates extent of acellularity

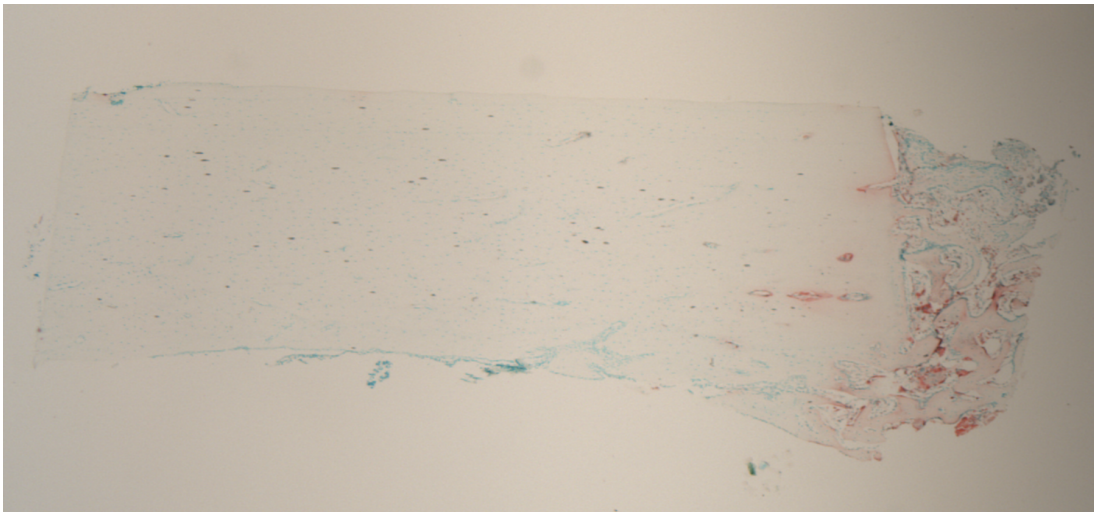

Figure 12A: TRAP Section: Control 2.5x Magnification. Red indicates osteoclastic activity.

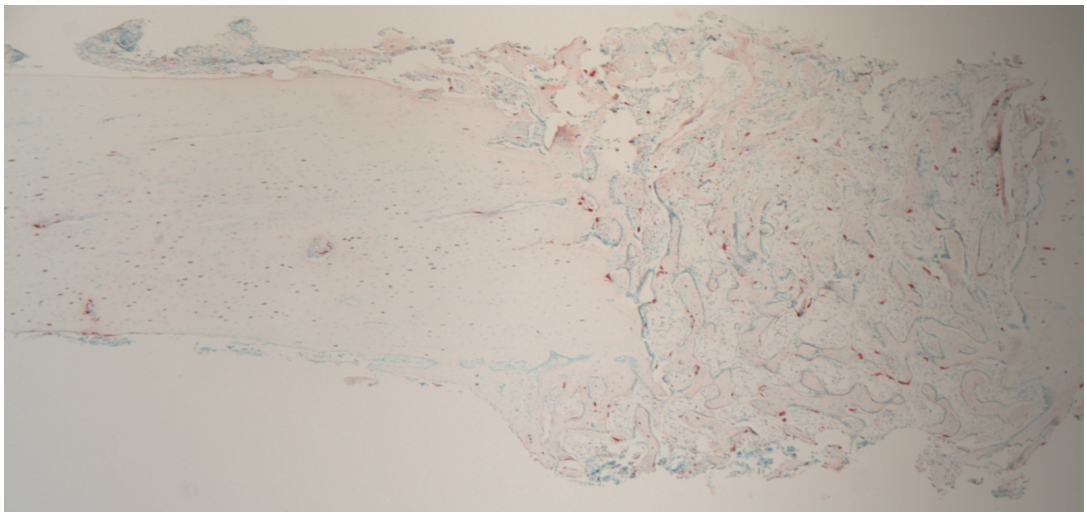

Figure 12B: TRAP Section: Experimental 2.5x Magnification. Red indicates osteoclastic activity.

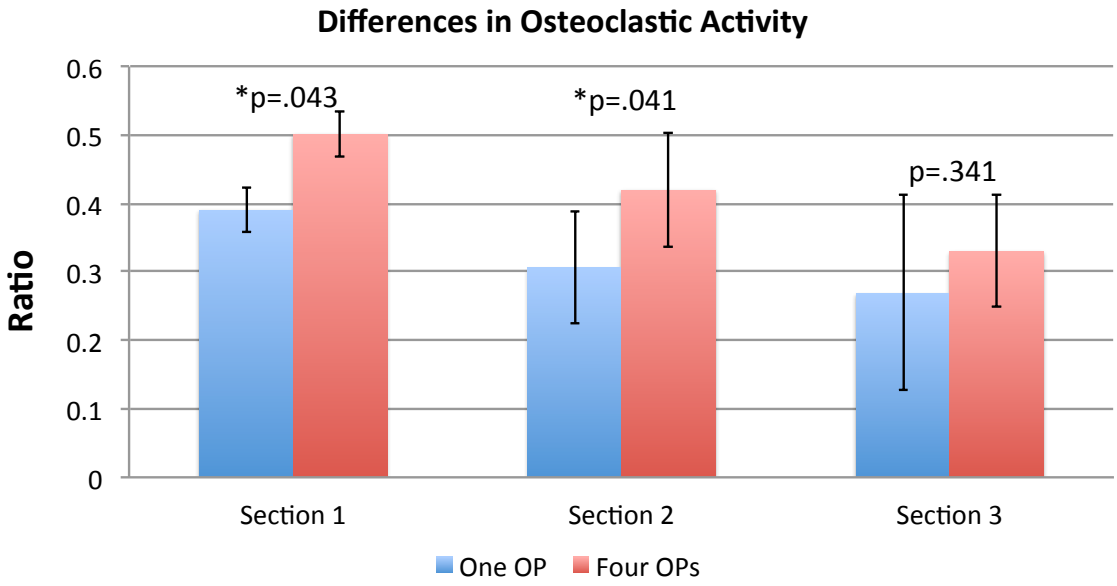

Figure 13: Differences in Osteoclastic Activity. Osteoclastic activity at each distance away from the osteoperforations. (*) indicates significance $p<0.05$.