THE EFFECTS OF CIRCUMFERENTIAL SUPRACRESTAL FIBEROTOMY (CSF) ON BONE AND GINGIVAL TISSUES IN BEAGLES

A Thesis

by

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ABSTRACT

Introduction: The purpose of this study was to determine whether performing a circumferential supracrestal fiberotomy (CSF) decreases the amount of surrounding dentoalveolar bone, and to evaluate the reorganization and healing of supracrestal gingival fibers following CSF.

Methods: Using a split-mouth design, CSF was performed on 2 maxillary teeth of 7 beagle dogs. The control side received no CSF. After either 2 or 4 weeks of healing, μCT was used to evaluate the quality and maturity of the dentoalveolar bone using bone density, bone volume, and trabecular thickness and number. Histologic analyses were performed to evaluate bone remodeling and healing of gingival fibers.

Results: μCT showed a significantly decreased (9%) bone volume fraction in the coronal bone sections of the experimental teeth. There was no significant difference in bone quantity apical to the crestal bone. TRAP staining showed an increase in TRAP activity along the surfaces of the crest of the alveolar bone, as well as in the lamina dura at two weeks. After four weeks, the TRAP activity had decreased to control levels. H&E and picro-sirius red stains demonstrated that the supracrestal gingival fibers were reattached but disorganized 2 weeks and 4 weeks after CSF. There was no difference in the fiber organization after 2 or 4 weeks.

Conclusions: The bone demineralization and remodeling associated with CSF is limited to the area immediately adjacent to the CSF. The demineralization event is

transient, lasting less than 4 weeks. The supracrestal gingival fibers reattach, but are still disorganized at 2 weeks and 4 weeks following CSF.

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NOMENCLATURE

CSF	Circumferential Supracrestal Fiberotomy
H&E	Hematoxylin and Eosin
MOP	Micro-osteoperforation
RAP	Regional Acceleratory Phenomenon
TRAP	Tartrate-resistant Acid Phosphatase

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The length of orthodontic treatment is a problem for both orthodontists and patients alike. The duration of comprehensive orthodontic treatment ranges from 21-27 months for non-extraction treatment, and 26-35 months if extractions are needed.[1] The length of treatment is often dependent on the rate of tooth movement. The typical rate of tooth movement is 1 mm per month.[2,3] If the teeth need to move long distances, this increases the length of treatment and consequently, the risks of gingival inflammation, root resorption, and white spot lesions on the enamel of teeth.[4-6] The duration of treatment is also heavily dependent on compliance by patients, as well as the number of broken brackets and missed appointments. Compliance decreases as treatment progresses, which further justifies finding ways to accelerate the rate of tooth movement and reduce the length of treatment.[7] Accelerating treatment would make it possible to decrease risk, meet the patients need for faster treatment, and still allow practitioners to provide uncompromised treatment results.

The following literature review will first discuss the biology of tooth movement, and then review the different procedures used to accelerate the rate of tooth movement and decrease treatment time. The biologic basis of accelerated tooth movement will then be examined and the review will conclude with literature pertaining to circumferential supracrestal fiberotomies (CSF), focusing on how this procedure could potentially be used to increase the rate of tooth movement in orthodontics.

Biology of Tooth Movement

With conventional treatment, orthodontists are limited by the biological processes that are responsible for tooth movement. In order to find ways of accelerating tooth movement, it is important to understand the basic biology of how teeth move. There are several proposed mechanisms, including the piezoelectric, pressure tension, and mechanotransduction hypotheses, that attempt to explain the bony changes that must occur in order for a tooth to move. While it is possible that the actual mechanism may be a combination of several hypotheses, mechanotransduction provides the most likely explanation for tooth movement.

Piezoelectric Hypothesis

The piezoelectric hypothesis was first made popular in the 1960s. As crystalline structures such as hydroxyapatite are deformed, the stress is converted to electric stimuli and there is a flow of electrons from one area of a crystal to another. This creates a change in electric polarity. Basset and Becker reported that bone under compression will develop negative potentials. [8] These electrical potentials can affect osteogenic tissues and stimulate bone formation. After the force is applied, the signal quickly dies away and an opposite signal is created when the force is removed, resulting in a reverse flow of electrons. These signals are likely important in the daily maintenance of bone during normal function. It has been demonstrated that when an electrical current is applied, teeth move faster than with conventional orthodontics alone.[9] However, with the sustained forces used in orthodontics, the signal would be short-lived, because the

signal would rapidly die after the initial application of force. As such, it is unlikely that this hypothesis alone is responsible for the mechanism of orthodontic tooth movement.

Pressure-tension

The pressure tension hypothesis is the classic theory of tooth movement.[10-12] This hypothesis proposes that when forces are placed on the tooth, part on the periodontal ligament will be compressed and part of the ligament will be stretched, under tension. This causes changes in the local chemical environment which stimulates cellular activity. Bone deposition occurs on the tension side and resorption occurs on the pressure side. With light pressure on the PDL, blood vessels are compressed and a decreased blood flow results. Cytokines and prostaglandins are released, as well as other messengers that help regulate osteoblast and osteoclast activity. Osteoclasts are activated to resorb bone with frontal resorption. With heavy forces and increased pressure on the PDL, a cell-free area of necrosis and "hyalinization" occurs. Osteoclasts must be activated from a distant area and resorb bone towards the tooth, resulting in undermining resorption. Undermining resorption occurs slower than frontal resorption. King et al, who evaluated histologic sections of alveolar bones in rats, reported increased bone deposition on the "tension side" and increased bone resorption on the "pressure side."[13]

Mechanotransduction

The mechanotransduction hypothesis proposes that the mechanical forces are converted to an electrical signal by osteocytes in the bone.[14] Osteocytes are connected to other osteocytes and bone surface lining osteoblasts via long slender processes

connected by gap junctions, forming a cellular network. The bone that surround the cells and their processes is not mineralized and is more easily penetrated by fluid, creating the lacunocanalicular porosity. When strain levels change on the bone, there are corresponding changes in fluid flow in the PDL and in the canaliculi. This creates a fluid shear stress that is sensed by the osteocytes, which act as mechanosensory cells, resulting in an increase in growth factors, matrix synthesis and gene activation. These charges and cellular signals regulate osteoblasts and osteoclast recruitment. These signals can be relayed through the osseous connected canalicular network which is connected by gap junctions. [15]

Rate of Tooth Movement

The average rate of tooth movement is approximately 1 mm per month.[3,16] However, rates vary greatly between and within individuals. There are qualities of the bone that can influence tooth movement including bone density and the rate of bone turnover.

Rate of bone turnover

Verna et al demonstrated that there is a relationship between bone remodeling and the rate of tooth movement in a study of 52 Wistar rats.[17] The rate of bone turnover was modified in the rats by pharmacologically induced hypothyroidism and hyperthyroidism, which was compared to a third control group. They confirmed that the levels of bone turnover in the 3 groups were significantly different. A constant mesial force was placed on a molar to cause tipping. They concluded that the amount of tooth movement was

significantly greater in the hyperthyroid group (high bone turnover) than the control rats, which were in turn greater than the hypothyroid group (low bone turnover).

Bone Density

Bone density has also been shown to significantly affect rates of tooth movement. In a study by Goldie et al, 35 rats were separated into two groups.[18] The first group had a dietary induced calcium deficiency and lower bone density; the second was a control group. They concluded that the rats with lower bone density had significantly faster tooth movement than the control rats.

Similarly, Ashcraft et al demonstrated the effects of decreased bone density by pharmacologically inducing osteoporosis in 13 New Zealand rabbits with corticosteroids, and compared these to a control group.[19] They mesialized the maxillary first molars for two weeks. The rabbits with decreased bone density had three to four times greater tooth movement than the controls. They also concluded that the decreased bone density resulted in faster tooth movement.

The Regional Acceleratory Phenomenon

In recent years there have been numerous methods proposed to accelerate the rate of tooth movement. These methods typically involve inducing trauma, which causes inflammation in the alveolus. This stimulates the regional acceleratory phenomenon (RAP), first described by Harold Frost in 1983. [20] The RAP is a complex and local reaction to noxious stimuli that results in the acceleration of the normal healing processes, accelerated bone turnover, regional decreases in bone density, increased perfusion, and increased cellular metabolism. Taking advantage of the RAP by injuring

bone has become common in orthodontics and is often called surgically assisted orthodontics.

Corticotomy

The RAP is usually achieved surgically with corticotomies involving a full thickness mucoperiosteal flaps and creating perforations or incisions in the bone. Corticotomies date back to 1959, when Heinrich Kole proposed that it worked as a variation of distraction osteogenesis, where blocks of bone are moved independently without waiting for PDL-mediated bone remodeling to occur. The technique was popularized by the Wilco brothers in 2001. The CT scans of their patients demonstrated significantly decreased bone density after the corticotomies, leading them to conclude that the increased rate of tooth movement was due to the RAP rather than movement of bone segments. The surgical technique that they used involved full thickness mucoperiosteal flaps with vertical corticotomies between the teeth connected subapically. The corticotomies were then covered with a bone allograft and the tissues were reapproximated. They reported a dramatic decrease in treatment duration, with some patients completed in as short as six months. However, they did not have control groups to compare treatment times.

Several studies have been performed that demonstrate increased rates of tooth movement following corticotomies. In a canine study by Cho et al in 2007, full thickness mucoperiosteal flaps and corticotomies were performed in the buccal cortex. The study had a very small sample size, including only two beagle dogs. The corticotomies were performed in one maxillary quadrant and one mandibular quadrant.

They found 4 times faster tooth movement in the maxilla and 2 times faster tooth movement in the mandible.[21] In 2009, Sanjideh et al found twice as much movement when protracting mandibular third premolars in foxhounds following buccal flaps with corticotomy ($p<0.05$).[22] In another canine split mouth study conducted by Iino et al in 2007, corticotomies were performed on the mandible of 12 beagles, and the third premolar was protracted.[23] They found 2 times faster tooth movement on the experimental side.

In a randomized controlled trial in 2014 by Fischer et al, 6 patients with bilaterally impacted canines were included in the split-mouth study. One of the canines had a full thickness mucoperiosteal flap, along with perforations along the mesial and distal lengths of the root, 2 mm apart and 1.5 mm deep. The other side had traditional canine exposures. The age range was 11-12.9 years and the patients were consecutively treated. A force of 60 g was placed on the tooth, which was monitored until it had reached the level of the occlusal plane. They found reduced treatment times ranging from 28-33% on the experimental side, a difference that was significant at the 0.001% level. [24]

Flapless Corticotomy

Although corticotomies have shown positive results, the mucoperiosteal flap causes alveolar bone loss and possible dehiscence. As a result of these and other possible risks of corticotomies, there has been a move toward less invasive methods for accelerating tooth movements. Other techniques for inducing the RAP, including micro-

osteoperforations and flapless corticotomy, have been investigated with varying levels of success.

Flapless corticotomies do not produce the same results as the classic corticotomies with a full thickness mucoperiosteal flaps. A split-mouth study of five beagle dogs by Safavi et al used nickel titanium closed coil springs to protract the second premolars.[25] Twenty-five small holes, 2 mm deep, were made without flaps in the cortical bone mesial and distal to the second premolars and in the first premolar extraction site. The decortication procedure was repeated after the first and second month, and the premolars were protracted for a total of three months. Although the rate of tooth movement was significantly greater during the first month of treatment on the piezocision side than the control side, it was less than on the control side during the third month of treatment. The overall difference was not statistically significant ($p=0.240$).

Micro-osteoperforations

Recently, micro-osteoperforations or MOPs have been used to increase the rate of tooth movement. This procedure perforates the buccal cortical plate with a miniscrew to cause the RAP with minimal risk or trauma. In a study by Cramer, MOPs were placed around a maxillary 2nd premolar of beagle dogs.[26] The premolar was distalized with a nickel titanium closed coil spring. He found that the tooth movement was slightly greater on the MOP side, but the difference was not clinically or statistically significant. The diminished result with flapless procedures demonstrate that the full thickness mucoperiosteal flap plays a role in inducing the RAP, as does the amount of injury.

Van Gemert et al studied the amount of damage cause by micro-osteoperforations (MOPs) on cortical and trabecular bone. They found that the density of the experimental bone was significantly less than the control bone, and the decreased density extended as far as 4.2 mm from the margin of the MOP. However statistically significant, the difference became much less at approximately 1. 5 mm from the MOP. TRAP staining showed increased osteoclastic activity 2 weeks after MOPs were placed, however by 4 weeks, the osteoclastic activity returned to control levels. Although a diffuse area of decreased density and increased osteoclastic activity could be seen, the effects were transient.

In 2012, Swapp et al studied the effects of using a bone awl to create 70 perforations in the cortex of 7 fox hounds. Using a split-mouth design, no flap was raised and the perforations made were 5-6 mm deep on one side of the jaw. They found no significant difference in the rate of tooth movement, concluding that the RAP was localized to the cortex and did not extend into the medullary bone.

Full-thickness Mucoperiosteal Flap and the RAP

A recent study by Owen et al in 2015 found that mucoperiosteal flaps alone, without any damage to the cortical bone, increases rates of tooth movement.[27] In a split-mouth design with seven beagle dogs, a full thickness mucoperiosteal flap was elevated on the buccal. The control side had no surgery. Mandibular second premolars were extracted, and a full-thickness mucoperiosteal flap was elevated, extending from the distal of the third premolar to the mesial of the first premolar. The tissues were reapproximated with sutures. The third premolar was protracted mesially with a NiTi

coil activated to 200 gm. Tooth movements were measured for 9 weeks after surgery. They found a 25% increase in the tooth movements on the flap side when compared to the control side. The increased tooth movements were the result of decreased medullary bone density. They also found a significantly decreased bone volume fraction mesial to the third premolar ($p<0.05$) in the experimental group. No differences were evident in the histologic sections. The increased rate of tooth movement was less than typically associated with corticotomy procedures,[21] but the decreased density in medullary bone was greater than that found in flapless corticotomy studies.[28] They did not find a significant difference in osteoclast or osteoblast activity, but it is possible that these effects would no longer be apparent 8 weeks after the surgery.

In a study by Yaffe et al in 1994, a sample of 60 Wistar rats were divided into three groups: a control group that received no surgical insults, a group that received a full thickness mucoperiosteal (FTMP) flap on the buccal aspect of the mandible, and a group that received FTMP flaps on both the buccal and lingual aspects of the mandible.[29] The flap procedure was performed with a small periosteal elevator and the tissue was reapproximated without sutures. High resolution x-ray microradiography was used to evaluate 1-1.5 mm sections of the mandible. Bone resorption was seen as early as 10 days after surgery. Greater amounts of resorption were seen in the group that had both buccal and lingual flaps. The maximum amount of resorption occurred 3 weeks after surgery. They also found resorption on both the periosteal aspect of the cortical bone, as well as on the PDL-aspect of the alveolar bone. Resorption might be expected because the periosteum provides 70-80% of the arterial blood supply and 90-100% of the venous

return in long bones. These studies suggest that disruption of the blood supply to the bone causes a superficial necrosis and stimulates the RAP extending into the medullary bone.

It has also been well documented that damage to the supracrestal fibers results in crestal bone loss. Binderman et al investigated the effect of mucoperiosteal surgery on the alveolar bone by comparing flaps raised from the marginal gingiva to an apical approach. [30] The sample consisted of two groups with 9 rats in each group. In one group, a mucoperiosteal flap was raised from a coronal approach and the marginal tissues were separated from the bone. In the second group the flap was raised from an apical approach, leaving the marginal tissues intact and attached to the crestal bone. The flaps were performed on both the buccal and lingual aspects of the mandible. The rats were sacrificed 21 days after the surgery. An analysis of bone loss was performed based on microradiography. Approximately 54% of the sections in the coronal approach group showed no bone loss, compared to 87% of those in the apical approach group. The coronal approach also had significantly more sections with partial or total alveolar bone height loss than the apical approach group. These results showed that disruption of the supracrestal fibers resulted in greater bone loss. There were group differences in the histological sections. The apical approach showed slight bone resorption on the outer surface of the bone. However, when the supracrestal fibers were removed in the coronal approach, extensive bone resorption was seen on the PDL aspect of the alveolus. There was also an increase in the number of osteoclasts, inflammatory cells, and necrotic bone with empty lacunae in the histologic sections of the coronal approach group. A full

thickness mucoperiosteal flap was performed in this study, which, as Owen et al demonstrated, causes changes in the alveolar bone by disrupting the vasculature. However, they additionally demonstrated that increased bony changes occur when the marginal gingiva was included in the flap. Although they qualified the bone resorption, especially in reference to the alveolar height, they did not objectively measure the extent of the bone resorption that occurred.

Circumferential Supracrestal Fiberotomy

The circumferential supracrestal fiberotomy (CSF) procedure was first introduced by John Edwards in 1970,[31] and the name was coined by Phillip Campbell in 1975 in an investigation of the effect of CSF on closure of midline diastemas.[32] In this surgical procedure, a number 11 Bard-Parker blade is inserted into the gingival sulcus along the root surface to sever all attachment surrounding the tooth to a depth of approximately 3 mm below the crest of the alveolar bone. CSF has classically been used in orthodontics to decrease relapse after correction of severely rotated teeth. While the osseous tissues reorganize relatively quickly following orthodontic treatment, the supracrestal tissues take much longer to reorganize and place a tensile force on the teeth. Using India Ink tattoos on the gingiva, Edwards demonstrated in a classic study that the supracrestal fibers have an elastic pull on the teeth. [31] The tattoos showed distortion after orthodontically rotating the teeth, indicating that the gingival fibers stretch and follow the movement of tooth. Following the CSF procedure, the gingival fibers were released from the tooth and the tattoo returned to its original shape within 20-40 hours, indicating that the tissues had relaxed. In his original publication on the topic, Edwards stated that

the surgical technique is simple and that there are few complications, making it easy to incorporate as a routine part of every orthodontist's retention therapy.

In a later study by Edwards, it was found that the relapse of teeth treated with CSF was significantly less than for control teeth.[33] Approximately 2-3 years post retention, the CSF group had 13.8% relapse, compared to the control group with 42.5% relapse. However, the difference in relapse between the two groups decreased 13-15 years posttreatment. They did not find any significant difference in the epithelial attachment between the two groups.

Supracrestal Periodontal Fibers

The transseptal fibers were described by Parker in 1972.[34] There are three types of "ligament-like" fibers that extend from the cementum of one tooth to the adjacent tooth, tooth to bone, and from the tooth into the surrounding connective tissue. These fibers are embedded in the cementum of the CEJ. The fibers that extend from cementum to the surrounding subepithelial connective tissue appear to have no mechanism of reorganization. Because of this they tend to relapse toward their original position when they are stretched with orthodontic treatment. In his study, Parker extracted first premolars in 7 macaque rhesus monkeys and distalized the second premolar.[34] He removed the supragingival tissues with a horizontal incision coronal to the mucogingival junction and a sulcular incision, leaving the periosteum intact. He found significantly less relapse in the experimental side. After 30 days, the tissues appeared adequately healed and completely reorganized.

It has been proposed that the elastic tissues surrounding the teeth take several months to reorganize and by severing the attachment to the tooth, the fibers can reattach to the tooth in a more relaxed position. In a study by Reitan, six teeth were rotated 50-70 degrees in a canine model.[35] The teeth were retained in a rotated position from 15-232 days. They evaluated the degree of fiber reorganization at different time points, and determined that fibers running perpendicular to the root were reorganized. Although fibers in the middle and apical region of the root had reorganized completely after 147 days, the marginal fibers were still only partially rearranged after 232 days. The supracrestal fibers were still stretched and displaced after 232 days. He proposed that although the PDL fibers can reorganize with remodeling in the bone, the supracrestal fibers that do not insert into bone have no mechanism of remodeling and, as a result, take much longer to reorganize.

CSF and the RAP effect

Since CSF is such a simple surgical procedure with very few complications, it would be an ideal method of inducing the RAP. There have been several studies that investigated the effect of CSF on the rate of tooth movement. Tuncay and Killany conducted a study in 1986 using a rat model, during which they evaluated the movements of the maxillary first molars after repeated CSF procedures.[36] Closed coil springs were ligated from the first molar to the incisors. On a randomly selected side, they performed CSF around the molar and along the crest of the edentulous ridge. The CSF procedure was repeated every 3 days to prevent scar tissue attachment to the molars. After 30 days, radiographic analyses showed 0.63 mm of tooth movement on

the fiberotomy side, compared to 0.51 mm on the control side. This difference was statistically significant. They attributed this difference to the decreased resistance of the soft tissue in the fiberotomy group; however, they did not perform any histological evaluations to determine the effects seen on the alveolar bone.

In 1983, Glenn evaluated the effect of CSF on rates of tooth movement.[37] In the split-mouth study, they banded the canines of five cats and performed CSF on one side of the maxilla. They used 3/16", 2 ounce elastics from the 3rd premolar to the canine to tip the canine distally. Additionally, a wedge of gingiva was removed from the distal of the canine. Each week measurements were taken, fiberotomies were repeated, and elastics were changed. After six weeks, the fiberotomy side showed twice as much distal crown movement compared to the control side. The difference was statistically significant ($p<0.001$). A histologic evaluation of the canine immediately after surgery showed that the transseptal fibers were not under tension. One week after surgery, the gingival and transseptal fibers appear to be reattached to the tooth, however scar tissue was present. They also observed that crestal bone height was decreased one week after surgery and noted that "surgically induced bone resorption" could have contributed to the difference in tooth movement. Although the results were significant, the amount of tooth movement varied considerably within the group.

Young et al in 2013 also demonstrated that CSF can result in an increased rate of tooth movement in rats.[38] A group of 34 Wistar rats were divided into 3 groups: fiberotomy, apical mucoperiosteal flap, and no surgery. In the two surgery groups, the left side did not have appliances placed. It served as the control side. In the fiberotomy

group, CSF was performed by surgically separating the supracrestal fibers around the maxillary first molars. A periosteal elevator was used to ensure detachment of the fibers. In the second group, they performed a full thickness apical flap. A NiTi wire was bonded to the incisors to produce buccal movement of the first molars. After 14 days, the appliance was removed. The results showed that the teeth moved twice as much in the fiberotomy group than in the control group and flap surgery groups. The fiberotomy group also showed significantly less relapse than the other two groups. They proposed that the detachment of the marginal gingival fibers caused a strain relaxation that triggered a cascade leading to resorption of the alveolar bone. Although resorption of alveolar bone was observed using microradiography, they did not quantify the amounts of resorption or increased osteoclastic activity that occurred.

Another in vivo study by Kalra and coworkers was carried out on humans.[39] They retracted maxillary and mandibular canines of 14 patients into 1st premolar extraction sites. There were 9 maxillary and 4 mandibular arches evaluated in this split mouth study. Fiberotomy was performed on one side with a scalpel. Space was closed with composite T-loops activated to 200 grams. After 90 days of space closure and two reactivations of the loops, the tooth movement was measured from casts. They found that the fiberotomy side had 2.5 mm of canine movement in maxillary arch and 2.04 mm of distalization in the mandibular arch. On the nonsurgical side, they found 2.14 mm of movement in the maxillary arch and 1.44 mm of movement in the mandibular arch. These differences were not statistically significant, but the sample was small. No histology could be done in this study.

Biologic Basis for CSF and the RAP

Several of the previous studies demonstrated increased rates of tooth movement were observed following CSF. This provides indirect evidence that the CSF has an effect that extends into the alveolar bone. There are some possible mechanisms that could explain this phenomenon. The first hypothesis was proposed by Glenn and Tuncay.[36,37] They postulated that forces build up and accumulate in the soft tissues during tooth movement with stretching and compression of the gingival tissues. These tissues could provide resistance to tooth movement. They believed that separating the gingival attachment would eliminate the soft tissue resistance and allow the tooth to move more freely.

Young, Binderman, and Yaffe proposed that the marginal gingiva is the key to resorption on the PDL aspect of the bone.[38] When the marginal gingiva is disrupted, there is an abrupt decrease in the physiologic strain on the gingival fibroblasts. This causes a morphologic change in the fibroblasts, with the cytoskeleton of the cells remodeling and the cells becoming rounder and less elongated.[40] This morphologic change activates a chain of signals that propagates osteoclast-mediated alveolar bone resorption, which may start with a cellular release of ATP. When the fibroblasts are injured, there is a rapid release of ATP into the extracellular environment and activation of the ATP cell membrane receptor P2X4 occurs.[41] The P2X receptors play a significant role in regulation of osteoblasts and osteoclasts.

An in vitro study by Binderman et al evaluated ATP release, calcium influx, and changes in human fibroblast cell shape after decreasing the strain on the cells. [41]

Compared to cells that were still under a physiological strain, they found significant decreases ($p<0.01$) in the lengths of the fibroblasts after the strain reduction. Using a ATP Bioluminescent Assay, they found a 10-fold increase in the level of extracellular ATP in fibroblasts that had a strain reduction when compared to the control fibroblasts ($P<0.001$). However, the ATP decreased to the control level after 60 minutes. They believed this release of ATP from fibroblasts is regulated by changes in cellular calcium levels. Adding Ionomycin, a substance that increases calcium influx, into the cells produced a similar significant release of extracellular ATP, supporting the idea that cellular influx of calcium stimulated ATP secretion from fibroblasts. When the cells were in a strained state, the levels in intracellular calcium were low. When the strain was reduced, there was dramatic influx of calcium into the fibroblasts ($p<0.01$). Gene expression of P2X7 was significantly upregulated in the reduced strain group when compared to the controls. They also found RANK-L to be highly expressed in the fibroblasts after strain reduction. RANK-L is an important regulator of osteoclast differentiation and activity.

Binderman et al postulated that the mechanism of bone resorption with injury to the marginal tissues may be similar to the result seen with orthodontic tooth movement.[40] As teeth move, the PDL is compressed from and physiological strain on the PDL decreases, resulting in site-specific resorption on the alveolar bone.

In 2010, Nishio and Nanci published a study that investigated the role of the proteins, odontogenic ameloblast-associated (ODAM) and amelotin (AMTN) in the formation and repair of the junctional epithelium. Both proteins are members of the

secretory calcium-binding phosphoproteins (SCPP) family. They are present in the junctional epithelium, and are only expressed during initial formation of the junctional epithelium, during tooth eruption, and during regeneration of the junctional epithelium when it is damaged. In their study, they performed gingivectomies on rats and used immunochemistry assay to evaluate the expression of these proteins during healing.[42] Removal of the gingiva and the junctional epithelium was completed in 30 Wistar rats using curettes on the left side. The contralateral side served as the control. The rats were sacrificed at 3, 5, 7, and 14 days post-surgery. At 3 days, they found that the oral epithelium had migrated toward the tooth and that, by day 5, a new junctional epithelium had started to form. By day 14, the junctional epithelium had reformed but was longer than the control side. They found that ODAM is present very early in wound healing, 3 days after gingivectomy, and is concentrated in the junctional epithelium. The SCPP family is integral to skeletal mineralization and is related to stabilization of calcium and phosphate.[43] It is possible that these proteins are involved with the bone resorption and decrease in bone density associated with separation of the marginal tissues.

The previous studies provide some insight into how trauma to the PDL fibers might affect bone remodeling. However, they do not provide any information about the extent of the remodeling that occurs, when it occurs or how it occurs. Moreover, these studies were carried out on small animals, with small surgical fields that make the procedures more difficult. Rat bone density is also very different from human bone, and the bony response seen is these procedures may be different that that found in humans. A canine model is more useful because it has similar characteristics to human bone and

dental structures.[44] Therefore, the purpose of this study is to determine if a RAP effect is seen in the alveolar bone following CSF, and to evaluate the amount and extent of remodeling observed surrounding the tooth. The study hypothesizes that there will be a decrease in bone density and an increase in osteoclastic activity in the bone surrounding a tooth following CSF due to the inflammatory effect. The purposes of this study are to investigate (1) the effect of circumferential supracrestal fiberotomy (CSF) on the surrounding bone, and (2) the healing of the supracrestal fibers following CSF. Our study would hope to determine if the resorptive phase is extensive enough to have an impact on the rate of tooth movement in orthodontics.

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CHAPTER II

PURPOSE AND SIGNIFICANCE

The length of orthodontic treatment varies from 21-27 months for non-extraction treatment, and 26-35 months when extractions are indicated.[1] Longer treatment times are associated with increased risks including gingival inflammation, root resorption, and white spot lesions on the enamel.[4-6] Accelerating orthodontic treatment would make it possible to minimize these risks, meet the patients need for faster treatment, and still allow orthodontists to provide uncompromised results.

Several studies have found that the average rate of tooth movement with conventional orthodontic mechanics is 1 mm per month.[2,3] Rates of tooth movement can be accelerated by inducing the RAP, which results in increased bone turnover, increased osteoclast activity, and increased rates of tooth movement. The most common technique used to induce the RAP is by performing a corticotomy with a full thickness mucoperiosteal flap. This results in tooth movements that are two times faster when compared to control teeth.[21,22] Several less invasive methods have been attempted including flapless corticotomy,[25] and micro-osteoperforation with smaller effects than when a mucoperiosteal flap is performed.[26,28] All of these techniques involve trauma to the alveolar bone.

Other studies have found that laying a soft tissue flap can also cause bone resorption and the RAP effect. Owen et al found that a FTMP flap alone results in faster tooth movements.[27,29] Binderman et al used a rat model to compare flaps raised from a

coronal approach, where the marginal tissues were separated from the bone, to an apical approach where the marginal gingiva was left intact.[30] When evaluating the differences in the alveolar bone, the apical approach sections showed slight bone resorption on the outer surface of the bone. However, when the supracrestal fibers were removed in the coronal approach, extensive bone resorption was seen on the PDL aspect of the alveolus. Young, Binderman, and Yaffe proposed that the marginal gingiva is the key to resorption on the PDL aspect of the bone.[38]

The circumferential supracrestal fiberotomy (CSF) procedure was introduced in 1970.[31] It is performed by inserting a Bard-Parker blade into the gingival sulcus of a tooth to sever the supracrestal attachment fibers. CSF is a simple procedure and would be an ideal method of inducing the RAP. Several studies have demonstrated faster tooth movement following CSF, however these studies were done on small animals with a smaller surgical field and bone density that is different from humans.[36-38] Although histology was conducted in these studies, they did not quantify the extent of the bone resorption or the increased osteoclastic activity. They also did not provide histology on the healing and reattachment of the supracrestal fibers. The purposes of this study are to investigate (1) the effect of circumferential supracrestal fiberotomy (CSF) on the surrounding bone, and (2) the healing of the supracrestal fibers following CSF. Our study would hope to determine if the resorptive phase following CSF is extensive enough to have an impact on the rate of tooth movement in orthodontics.

CHAPTER III

MATERIALS AND METHODS

Sample

Seven skeletally mature male beagle dogs were used in the experiment. Each dog was approximately 2 years old and weighed 22-30 pounds. The experimental protocol, housing and care was approved by the Institutional Animal Care and Use Committee at the Texas A&M University College of Dentistry (IACUC 2016-0214-BCD). The dogs were fed a soft food diet throughout the experiment and maintained good overall health. All dogs were initially quarantined for 10 days prior to beginning the experiment.

Experimental Protocol

Prior to each procedure, the animals were fasted for 12 hours and sedated with a mixture of Ketamine (2.2 mg/kg) and Xylazine (0.22 mg/kg) administered intramuscularly. Dental prophylaxis was performed using an ultrasonic scaler with a chlorhexidine gluconate (0.12%) solution. Initial records included periapical radiographs taken of the maxillary posterior teeth using a size 4 phosphor plate. Vital signs were monitored during the procedure, and sterile conditions were maintained.

Following prophylaxis and radiographs, a circumferential supracrestal fiberotomy (CSF) was performed on a randomly chosen maxillary canine from one quadrant and the contralateral 2nd premolar. The contralateral counterparts of these two teeth served as controls. Four of the dogs had CSF performed at four weeks prior to

sacrifice (Day 1 of experiment). The remaining three dogs had CSF completed at two weeks prior to sacrifice (Day 14 of experiment).

The gingiva of each surgical site was anesthetized using gingival infiltration with 2% Lidocaine 1:100,000 Epinephrine (Patterson Dental, St. Paul, MN). The CSF was performed with a Bard parker and a #15 blade. A sulcular incision was used to sever the soft tissue attachment to the level of the crestal bone circumferentially around the tooth (Figure 1). The blade was inserted as far as the width of the blade would allow (approximately 1 mm below the crest of the alveolar bone). A small periosteal elevator was used to confirm that the attachment had been severed from the tooth.

All animals were sacrificed on day 28 of the experiment. The animals received an intramuscular injection of Ketamine (8-24 mg/kg) and Xylazine (0.22 mg/kg). Surgical plane anesthesia was confirmed. The animals were sacrificed by cannulating the common carotid arteries and administering 2 mL of Beuthanasia-D intracardially. The cannulas were flushed with 1.5 liters of saline solution and 1 liter of 4% paraformaldehyde (PFA). The maxilla was harvested and stored in 4% PFA. The maxilla was sectioned into blocks to include the bone distal to the canine, mesial to the second premolars, and distal to the second premolar (Figure 2a). The samples were diluted to 0.5% PFA prior to micro-CT.

Data Collection and Analyses

Micro-CT

Micro-CT was performed to evaluate the density and maturity of bone immediately adjacent to the teeth. Blocks that included the canines and bone distal to

the canines, as well as block that included the second premolars and bone mesial to the second premolars, were analyzed (Figure 2b). The block specimens were placed in 20 mm wide micro CT tubes, stabilized with foam, submerged in 0.5% PFA and sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, IL). The samples were scanned with a ScanCo Medical Micro-CT 35 (ScanCo Medical, Bassesdorf, Switzerland) at a resolution of 30 μm, 55 kVp voltage, 145 μA current, and 600 ms integration time.

For each block, the maturity and density of two areas of alveolar bone were evaluated, including a large wide-spread volume and a small coronal volume. The borders for the large volume were limited sagitally to include the bone immediately adjacent to the tooth of interest and extended to the adjacent tooth, avoiding the lamina dura of the adjacent tooth. Vertically the large volume included the middle 60% of the bone to the experimental tooth. The middle 60% of the root length was determined by locating the slice where the apex began, as well as the slice where the alveolar bone crest began, and removing 20% of the slices at each end. In the small coronal volume, the area of interest was limited to the bone immediately adjacent to the experimental tooth and extended 1 mm horizontally from the tooth's root surface. The bone volume was limited vertically to include only the coronal 25% of the bone extending from the alveolar crest. The threshold boundaries for the scans were set from 300 to 1000 Houndsfield units for the samples.

Histology

The samples were evaluated using hematoxylin and eosin stains (H&E), and TRAP (Tartrate-resistant acid phosphatase) stains. Additionally, picro-sirius red stain was used to visualize collagen fibers. The samples were fixed in a 4% PFA solution, and decalcified in EDTA. They were then dehydrated with a series of alcohols, cleared with xylene, and then infiltrated and embedded in paraffin. Samples were oriented and sectioned in a sagittal plane with a thickness of 30 microns. These sections were mounted onto glass slides and stained. The H&E and TRAP slides were viewed and photographed under a Zeiss Axioplan microscope ICarl Zeiss Microimaging, Germany). The picro-sirius red slides were viewed under an Olympus BX51 microscope with polarized light and photographed using an Olympus DP72 camera.

Statistical Analysis

IBM SPSS statistics for windows (Version 23. Armonk, NY: IBM CorpL) software was used to compare groups and describe the results. Data from the uCT were analyzed using means and standard deviations after the data was determined to be normally distributed. Paired t-tests were used to analyze group differences between experimental and control specimens, using 1-tailed tests and a significance level of $p<0.05$.

CHAPTER IV

RESULTS

Following surgery, healing of the gingival tissues progressed normally with no swelling and no signs of infection in any of the animals. Gingival tissues healed within a few days of the procedure.

Micro-CT

The coronal 25% of inter-radicular bone adjacent to the experimental teeth had a significantly ($p=0.044$) lower bone volume fraction than the same bone adjacent to the control teeth (Figure 3A). Apparent density was also less on the experimental than control side, but the difference was not statistically significant. There was no between-side difference in material density (Figure 4A). There also were no between-side density or bone volume fraction differences in the middle 60% of the adjacent bone (Figures 3B,4B). When the samples were split into groups of 2 weeks and 4 weeks, no differences were found in density or bone volume fraction. This is likely due to lack of power associated with the small sample sizes.

When the 2 week and 4 week samples are combined, both the coronal 25% and middle 60% sections show significantly ($p=0.028$, $p=0.008$) greater numbers of trabeculae than the same bone adjacent to the control teeth (Figure 5A, B). The coronal 25% showed thinner trabeculae as well when the 2 week and 4 week samples are combined ($p=.034$). There was no difference in trabecular separation between the two sides in either the coronal or middle sections ($p=0.112$, $p=0.198$).

When evaluating the 2 week samples alone, the same increase in number of trabeculae are seen in both the coronal and middle sections, but the difference is not significant (Figure 6A, B). There were also no significant differences in trabecular thickness or trabecular separations.

The experimental samples from 4 weeks post-CSF displayed significantly greater number of trabeculae in both the coronal and middle sections ($p=0.009$, $p=0.045$, Figure 7A, B). The coronal sections also show significantly ($p=0.001$) decreased trabecular thickness as well after 4 weeks (Figure 7A). There was no difference in trabecular separation.

Histology

H&E Staining

H&E sections showed that the gingiva around the experimental teeth was reattached to the root surface, but still disorganized 2 weeks and 4 weeks after CSF (Figure 8). The control teeth demonstrated well organized fibers that extended from the cementum to the gingival crest (Figure 8), horizontally towards the adjacent tooth, and inferiorly toward the alveolar crest. These distinct fibers were not seen in the experimental sections. There was no difference in the organization of the supracrestal fibers between 2 weeks and 4 weeks after CSF (Figure 9).

The experimental sections showed bone resorption along the lamina dura, with multinuclear osteoclasts lining the bone surface. The bone surface of the experimental teeth was often irregular with Howship's lacunae present. This was in contrast to the

generally smooth surface of the control bone of the lamina dura and on the alveolar crest (Figure 8).

Signs of inflammation were present in the sulcus and the gingival epithelium of both the experimental and control teeth. Large rete pegs or ridges, widening of the epithelium, and a large polymorphonuclear leukocyte infiltrate, were evident on both sides. The extent of inflammation was inconsistent throughout the specimens and there were no differences between sides.

The post-mortem CSF specimens confirmed that the supracrestal fibers were severed slightly past the crest of the alveolar bone (Figure 10).

TRAP Staining

Two weeks after CSF, the experimental bone stained with TRAP showed greater osteoclastic activity than the control bone (Figure 9). The experimental bone showed significant TRAP activity on the crest of the alveolar bone, as well as in the lamina dura. These areas of TRAP activity were diffuse and spread along surfaces of the bone. The control teeth showed normal TRAP activity lining blood vessels, and in some areas of the bone surface, but the TRAP activity was localized to small areas. There was little or no TRAP activity around the experimental teeth 4 weeks after CSF (Figure 10).

Picro-sirius Red Staining

Two weeks after CSF, the supracrestal fibers had reattached to the root surface. However, the fibers had not reorganized (Figure 11). Four weeks after CSF, the fibers were still disorganized (Figure 12). The collagen fibers following CSF were thinner on

the experimental than the control side after both two weeks and four weeks, and the thick cords of collagen were absent (Figure 11).

CHAPTER IV

DISCUSSION

CSF temporarily decreases the amount of bone surrounding the tooth upon which the procedure is performed. Analysis of μCT data demonstrated that following CSF, bone resorbed and bone density decreased, primarily in the crestal regions. Due to the limited treatment effect, when the samples were divided into two and four week groups, no statistically significant between group differences were found. This was likely due to lack of power associated with small sample sizes. However, when the 2 and 4 week samples in the present study were combined, the experimental group showed a significantly smaller bone volume fraction. Decreased bone volume fraction was found only in the most crestal bone adjacent to the tooth localized to the site nearest the soft tissue injury, extending approximately 1 mm horizontally from the root surface and 1 mm vertically from the alveolar crest. Bone further than 1 mm from the root surface, or further apically from the crest did not exhibit decreased bone density.

CSF appears to have a lesser effect on bone density than when the bone is directly injured. Decreased bone density has been reported 2 weeks after the placement of MOPS, extending up to 4 mm away from the MOP sites, which is much further than the effects identified in the present study. There was a 10-14% difference in bone density between the experimental and control groups immediately adjacent to the MOP site, [45] compared to a 9% difference in the present study. The lesser effects on bone in the current study is due to the fact that the surgical injury was limited to the soft tissue

adjacent to the bone, and did not directly damage the bone as is done with MOPs. Based on the relationship that has been established between the amount of trauma and extent of bone demineralization, the surgical insult to the bone produced by CSF must have been less than the insult produced by MOPs.[46]

The effects of CSF are limited to the bone surfaces. The TRAP and H&E stains in the present study showed increased cellular activity and bone resorption on the surface of the alveolar crest and in the lamina dura close to the crest of the bone, with minimal activity deeper in the trabecular bone. This is because the bone itself was not injured directly. Previous studies have shown greater alveolar bone loss on the PDL aspect of the buccal plate when a flap includes the supracrestal fibers than when there is no flap or when a flap does not include the supracrestal fibers.[30] Young et al found that 27% of alveolar bone sections showed bone resorption following CSF, compared to 12% in apical flap groups.[38] However, these studies were also done in rats, where the surgical procedure would have resulted in much greater surgical injury than in the current study. It has also been shown that a full thickness mucoperiosteal flap where the periosteum is raised causes a much greater injury to the soft tissue and disrupts the local blood supply to the bone, which results in resorption in the bone.[27] Van Gemert et al found that when the bone is directly injured with MOPs, TRAP activity increases up to 2.5 mm away from the MOP sites, with diffuse TRAP activity occurring throughout the affected area.[45] They also demonstrated that MOPs cause areas of bone necrosis and microfractures that extended from the MOP site. The comparison of these two studies show us that a localized injury to the supracrestal fibers of the gingiva does not cause as

much remodeling of the bone as when the bone is damaged directly. A rat study evaluating the effects of piezocision found extensive bone resorption extending up to 2.5 mm from the surgical site, with TRAP activity on the lamina dura and in the medullary bone, again demonstrating that damage directly to the bone itself causes significantly more bone resorption. [47] In the current study where only the supracrestal soft tissues are damaged, the effects were limited to the surface of the bone adjacent to where the supracrestal fibers were detached.

New woven bone begins to form shortly after CSF. When the two and four weeks experimental samples were combined, they showed increased numbers of trabeculae and a decreased thickness of the trabeculae. This is indicative of newly formed woven bone being laid down. We can speculate that bone was laid down on the surface following bone resorption, although fluorescent labeling would be needed to demonstrate the location of bone formation. The μCT data demonstrates that while the 2 week samples do not show any differences, both the coronal and middle sections of the 4 week samples showed significant increases in the number of trabeculae. After the bone is resorbed on the surface of the lamina dura and the alveolar crest, bone forming cells start producing new, immature bone. This process appears to start by 2 weeks, but becomes more evident after 4 weeks.

Previous studies investigating alveolar bone healing in dogs show a similar time course. Cardaropoli et al found that extraction sockets areas first filled with a coagulum, which is replaced by a provisional matrix of mesenchymal cells, leukocytes, and collagen fibers by day 7. By day 14 woven bone starts to form throughout the extraction

socket. Van Gemert et al concluded that remineralization and newly formed woven bone start appearing as early as one week after MOP placement.[45] Berglundh found that new woven bone starts to form in the first week after implant placement.[48] After 2 weeks of healing, the new bone formation was more widespread and extended around most implant surfaces.

The effects of CSF on bone resorption are transient. While increased TRAP activity was evident along the surface of the bone near the alveolar crest after two weeks of healing, it had decreased to control levels 4 weeks after CSF. This indicates that the limited RAP caused by CSF is transient and that the bone demineralization event lasts less than 4 weeks. This follows the standard model of bone healing. A similar timeline of demineralization is seen following MOPs.[45] Van Gemert et al reported increased TRAP activity in bone extending several millimeters away from the MOP after two weeks of healing, but not after four weeks. In a rat study evaluating the effects of piezocision found increases in the number of osteoclasts as soon as 1 day after surgery, which continued for 7 days, and then they steadily declined to baseline levels.[47]

CSF causes a loss of the large organized collagen fibers in the crestal soft tissues. In the current study, neither picro-sirius red staining or H&E showed large organized bundles of collagen on the experimental side. They were only seen on the control side. This indicates that the bundles had been damaged and remodeled away. Smaller, disorganized collagen fibers were evident. In the post-mortem CSF, the thicker organized bundles are still visible, and are clearly severed adjacent to the experimental tooth. Over the first 2 weeks post-injury, it is likely that matrix metalloproteinases

produced by fibroblasts remodel the damaged thicker bundles. Fibroblasts then start replacing them with smaller bundles as they reattach to the tooth surface. There was no apparent difference in organization of the supracrestal fibers after 2 and 4 weeks of healing. Rochester also found less organized supracrestal fibers following 8 weeks of healing after CSF, however the tissues did appear to have more mature collagen fibers than in the current study.[49]

The supracrestal fibers quickly reattached to the root surfaces. Several of the H&E and picro-sirius red sections demonstrated that the gingival fibers had reattached to the root surface 2 weeks after CSF. Although some of the control samples were torn during histology preparation, far more of the experimental samples were torn at the gingival margin. This may indicate that the attachment to the root surface was weaker in the experimental samples. Edwards noted that the tissue repair and healing was clinically complete 5-7 days after CSF.[31] However, the present study lends credence to the necessity for retention following CSF to provide time for complete fiber reattachment. Glenn et al found that the gingival fibers were fully reattached one week post-CSF on the mesial aspect and partially reattached on the distal aspect of teeth being tipped distally.[37] However, the histology shows that the reattachment is weak and easily compromised for up to 4 weeks post injury. No clinically significant changes in pocket depth have been reported 4 months after CSF.[50]

Clinical Significance

CSF should not be performed with the intention of stimulating the RAP effect. Due to the very limited effects of CSF on the bone density, in terms of both area and duration, only a very minor RAP effect is to be expected. With most of the bone resorption occurring at the most crestal aspect of the alveolar bone, one could speculate that the procedure may produce greater tooth movement due to tipping. Glenn et al reported that the center of rotation moved apically following repeated CSF during tooth movement, indicating more tipping movements of the teeth.[37] Rochester found that when CSF was performed only once, there was no significant difference in the rate or total amount of tooth movement between the experimental and control sides.[49] There was an increased amount of tipping in the experimental side, but the difference was not statistically significant. Clinically, since the supracrestal fibers are attached but not totally reorganized, retention following the CSF procedure used to prevent relapse following correction of rotations is indicated.

CHAPTER VI

CONCLUSIONS

1. Circumferential supracrestal fiberotomy temporarily decreases the amount of bone surrounding the tooth upon which the procedure is performed.
2. The slight demineralization of bone is limited to the superficial surfaces of the alveolar crest and the lamina dura.
3. The demineralization of bone following CSF is transient.
4. New woven bone begins to form shortly after CSF.
5. CSF causes a loss of the large organized supracrestal collagen fiber bundles, and the tissues were attached but were not reorganized after 4 weeks of healing.

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APPENDIX A

FIGURES

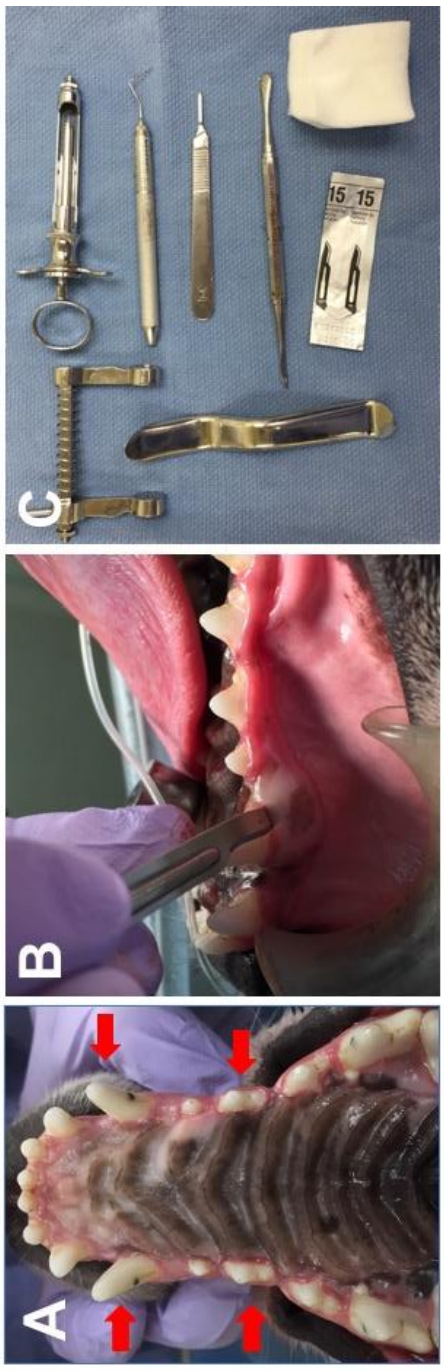

Figure 1. Surgical procedure. Experimental teeth included maxillary canines and 2nd premolars. (B) Blade was used to sever the periodontal attachment to the level of the crestal bone. The blade was walked 360 degrees around the tooth. (C) Surgical armamentarium included a Bard-Parker, #15 surgical blade, and a periodontal elevator

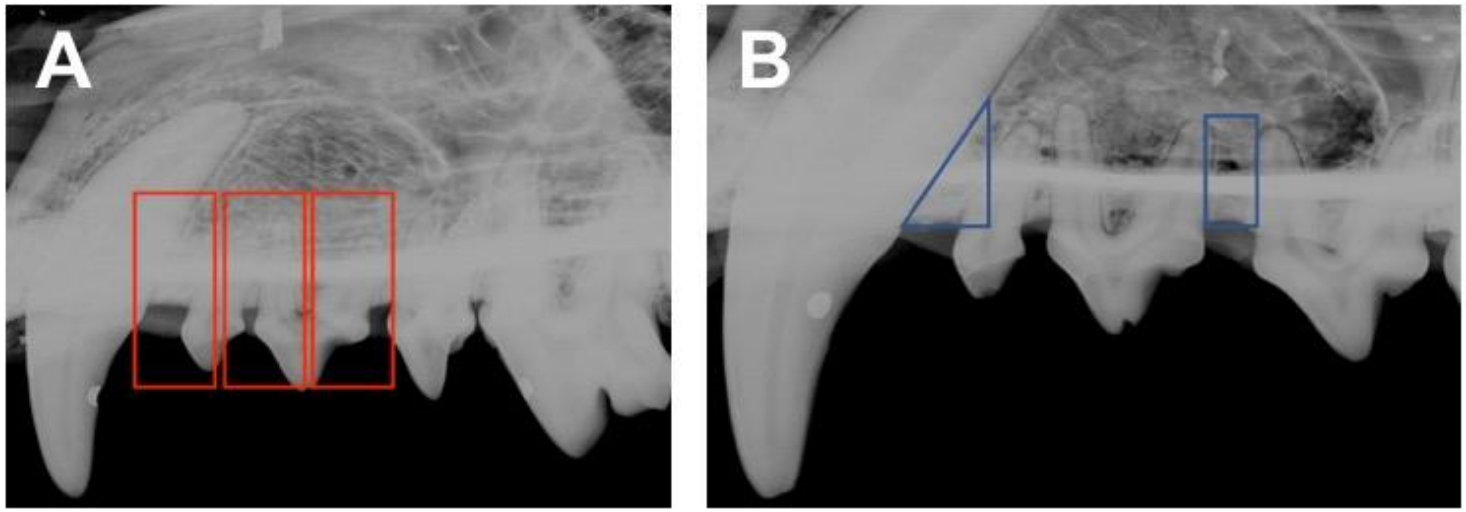

Figure 2. Sectioning of maxilla. (A) Sections for histological analysis (B) Sections of bone analyzed with micro

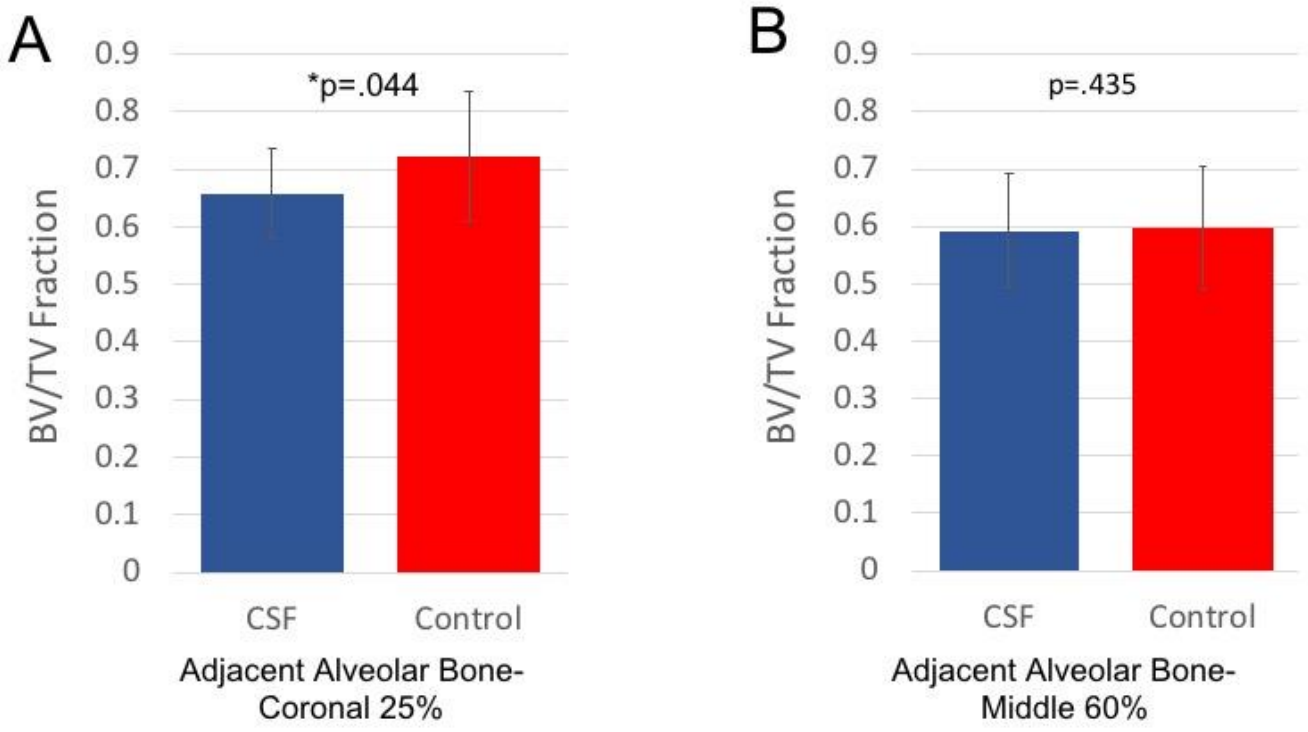

Figure 3. Bone volume fraction (BV/TV). Differences in bone volume fraction of the adjacent alveolar bone, along with standard deviation and probability (p, 1-tailed) of between/side differences. (A) Coronal section of the alveolar bone (B) Middle section of alveolar bone

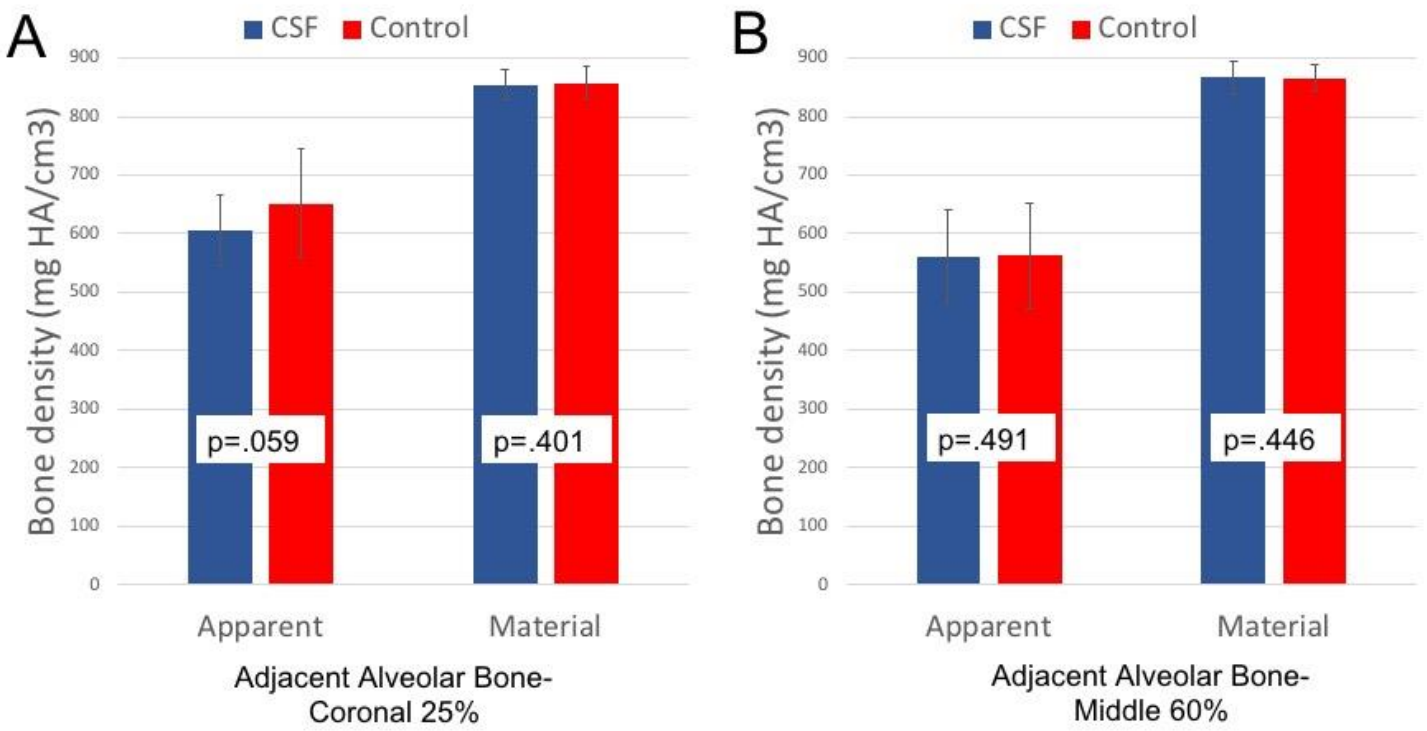

Figure 4. Apparent density and material density. Differences in apparent density and material density of the adjacent alveolar bone along with standard deviations and probability (p, 1-tailed) of between/side differences. (A) Coronal section of the alveolar bone (B) Middle section of the alveolar bone

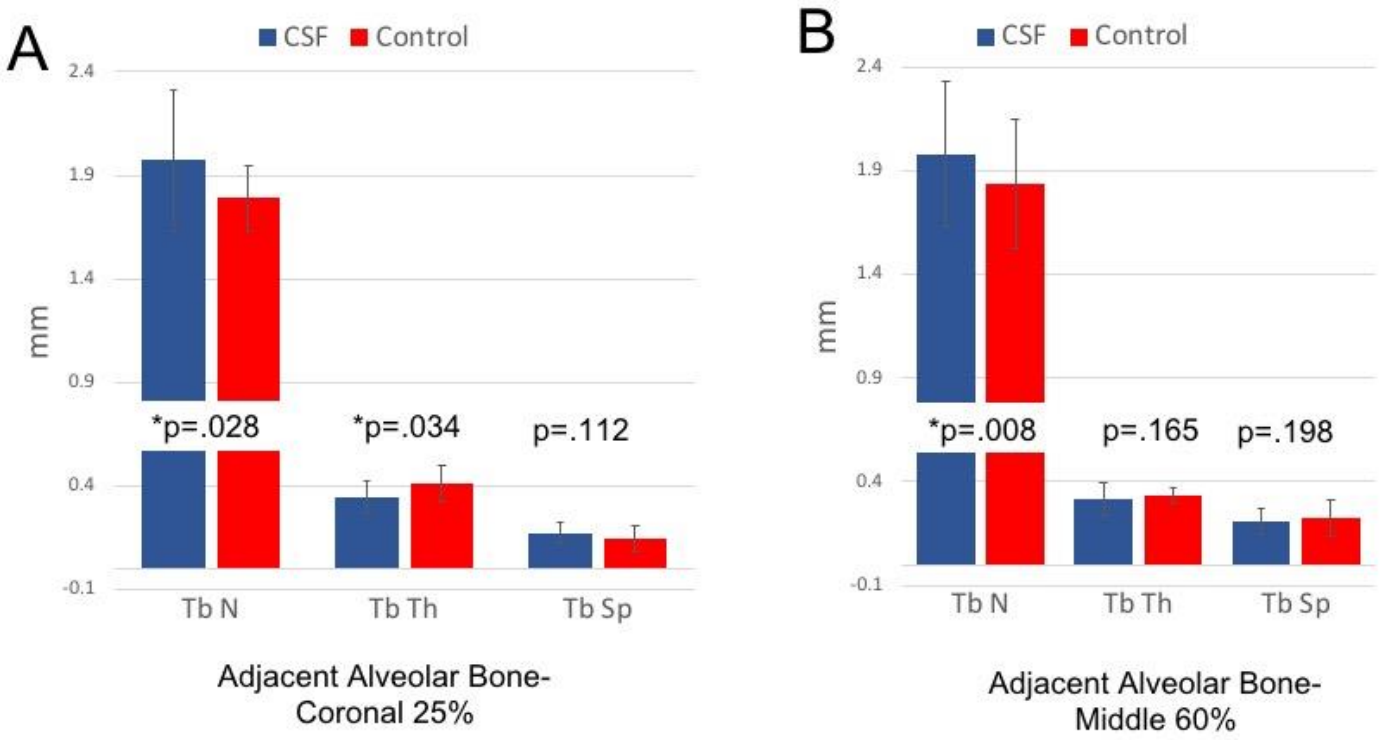

Figure 5. Differences in trabecular bone. Differences in number of trabeculae (Tb N), trabecular thickness (Tb Th), and trabecular spacing (Tb Sp) of the adjacent alveolar bone and the probability (p, 1-tailed) of between/side differences. (A) Coronal section of the bone (B) Middle section of the bone

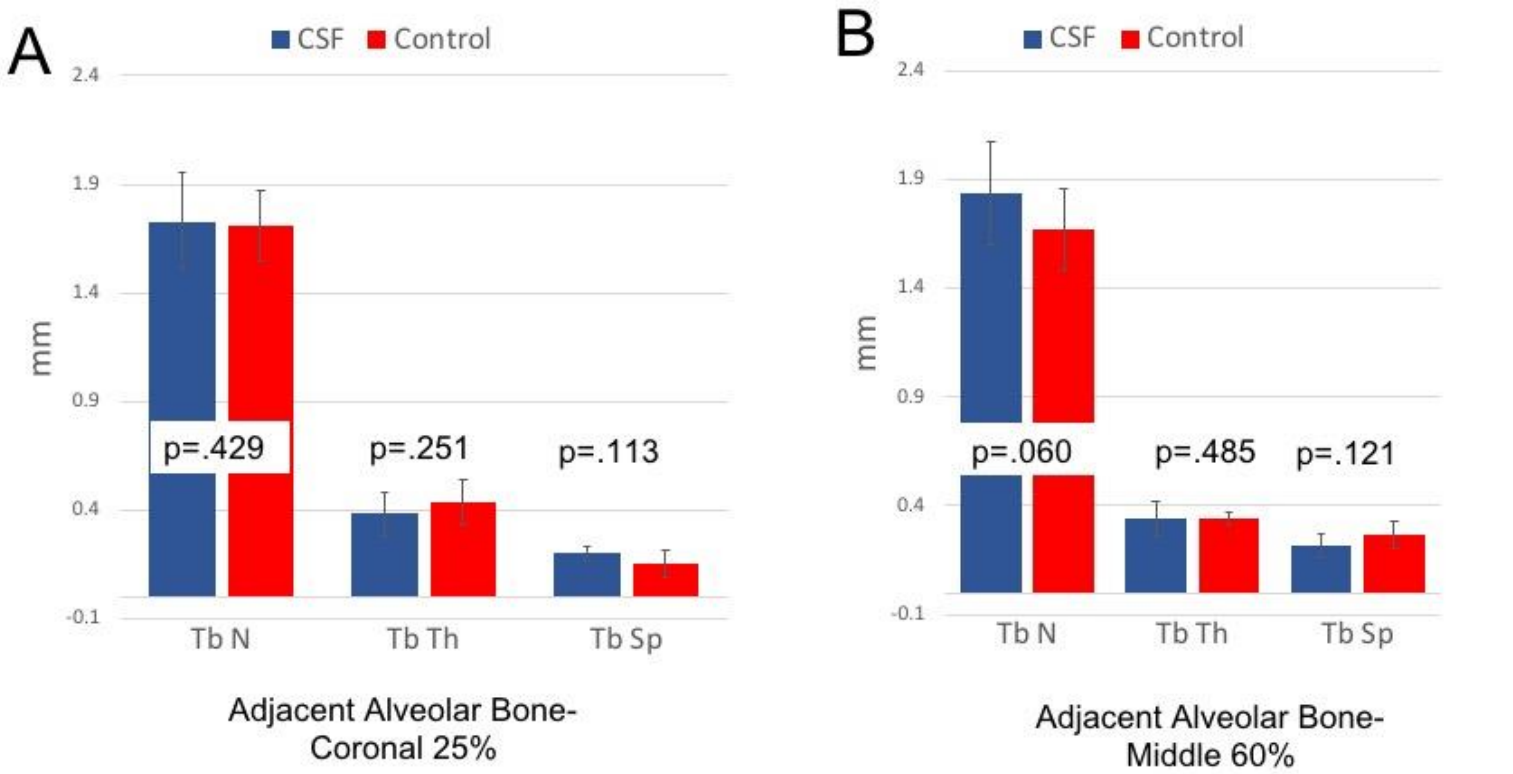

Figure 6. Differences in trabecular bone after 2 weeks. Differences in number of trabeculae (Tb N), trabecular thickness (Tb Th), and trabecular spacing (Tb Sp) of the adjacent alveolar bone and the probability (p, 1-tailed) of between/side differences. (A) Coronal section of bone (B) Middle section of bone

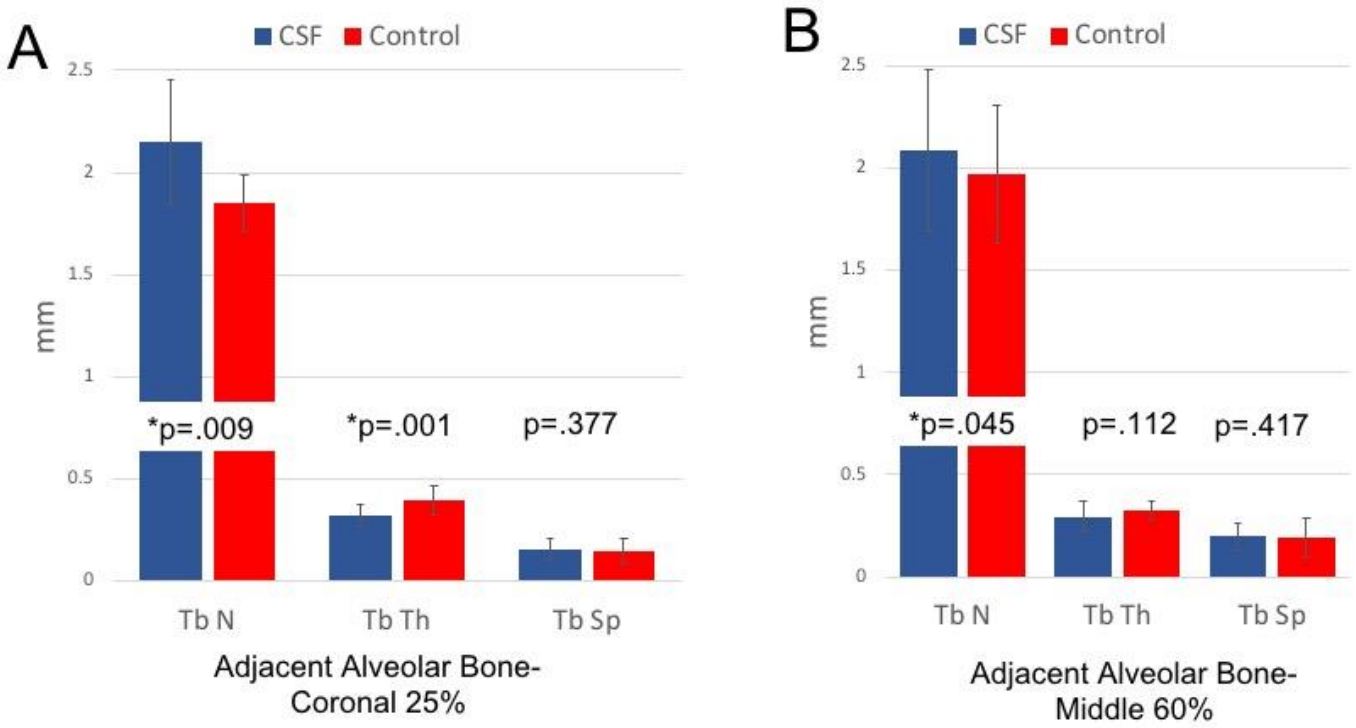

Figure 7. Differences in trabecular bone after 4 weeks. Differences in number of trabeculae (Tb N), trabecular thickness (Tb Th), and trabecular spacing (Tb Sp) of the adjacent alveolar bone and the probability (p, 1-tailed) of between/side differences. (A) Coronal section of bone (B) Middle section of bone

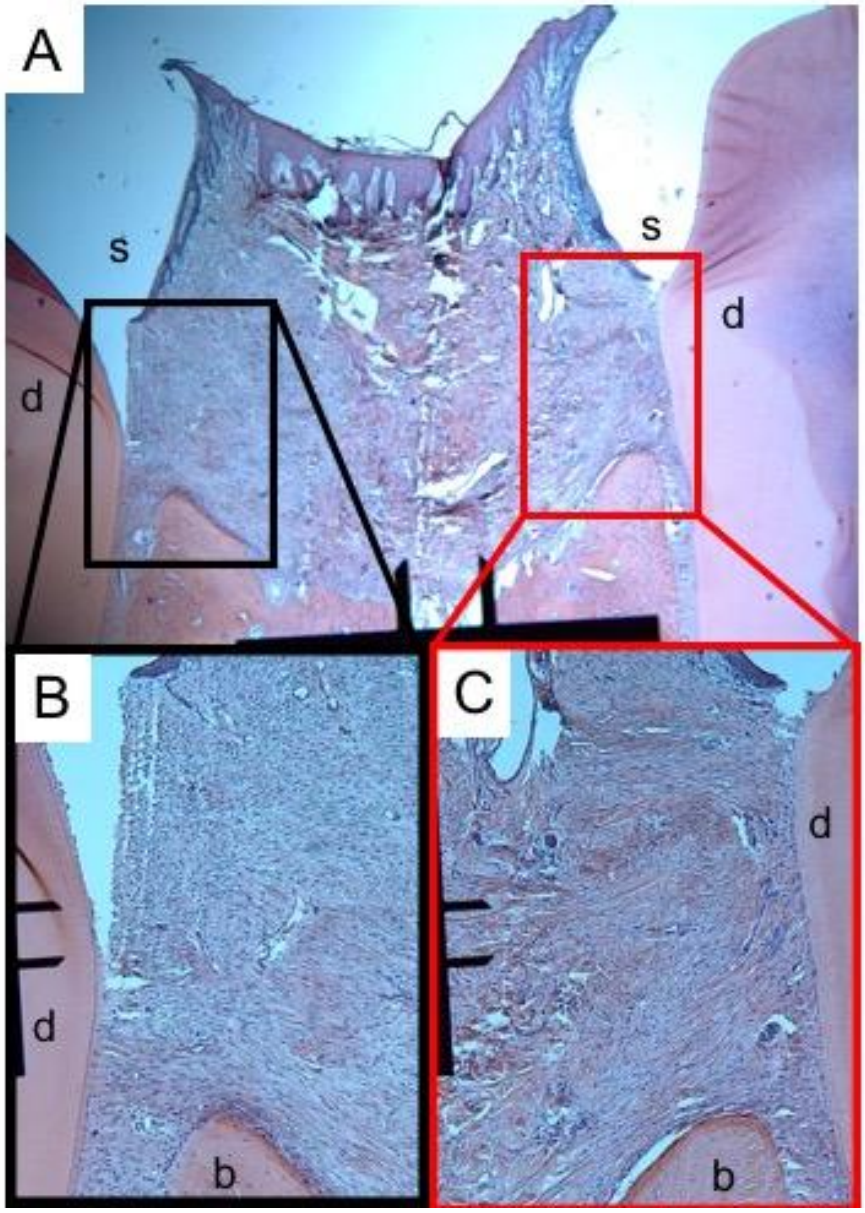

Figure 8. H&E: Supracrestal fibers following 2 weeks of healing. (A) 2.5X magnification of supracrestal fibers (B) 10X magnification of CSF side (B) 10X magnification of control side. Note disorganization of the collagen fibers on the CSF side after 2 weeks of healing. Control side without CSF shows distinct, organized collagen fibers. b=bone, d=dentin, s=gingival sulcus

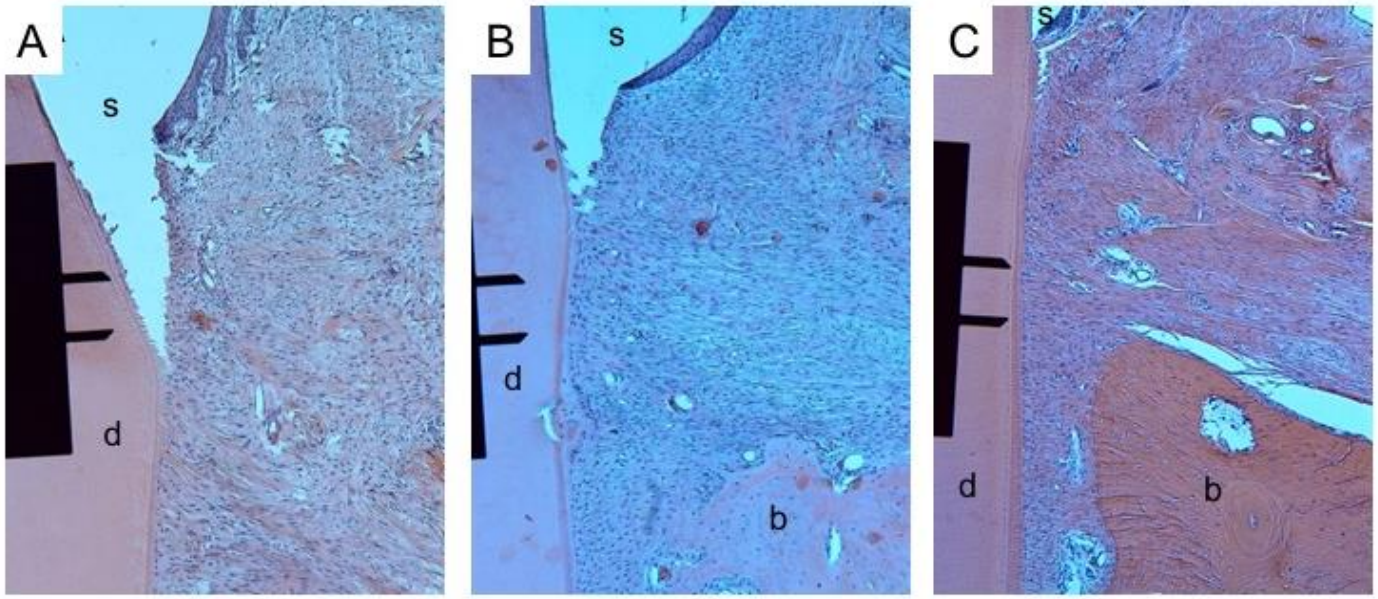

Figure 9. H&E: Comparison of supracrestal fibers following CSF after 2 weeks and 4 weeks of healing. (A) 2 weeks after CSF (B) 4 weeks after CSF (C) 4 week control. After four weeks of healing, the supracrestal fibers are still disorganized, lacking the thick organized collagen fibers. There does not appear to be a difference in the organization after two weeks and four weeks of healing. b=alveolar bone, d=dentin, s=gingival sulcus

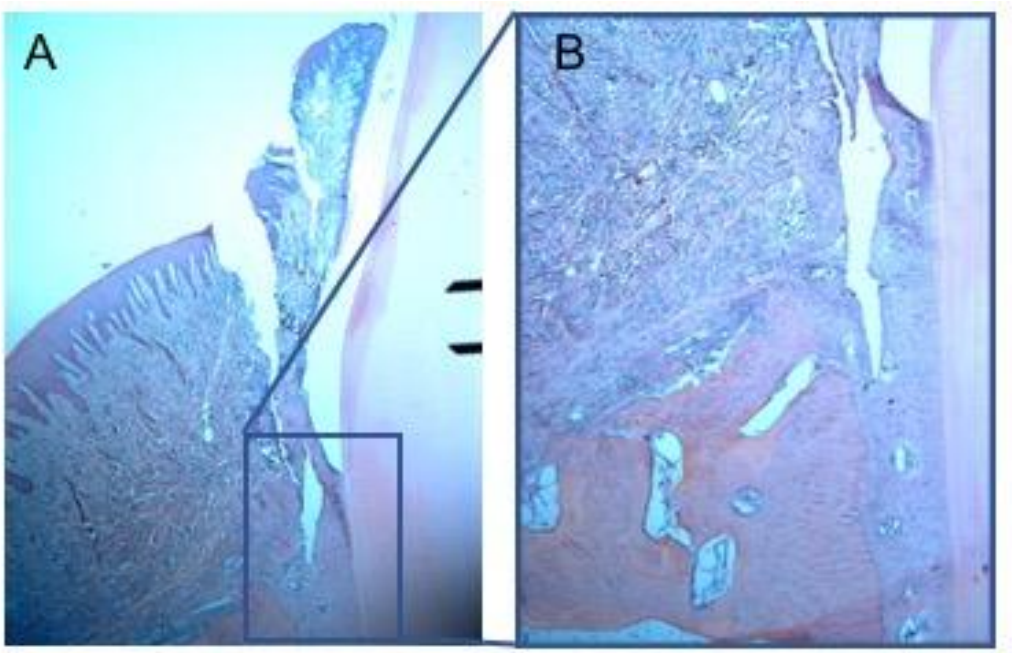

Figure 10. H&E: Post-mortem CSF. (A) 2.5X magnification (B) 5X magnification

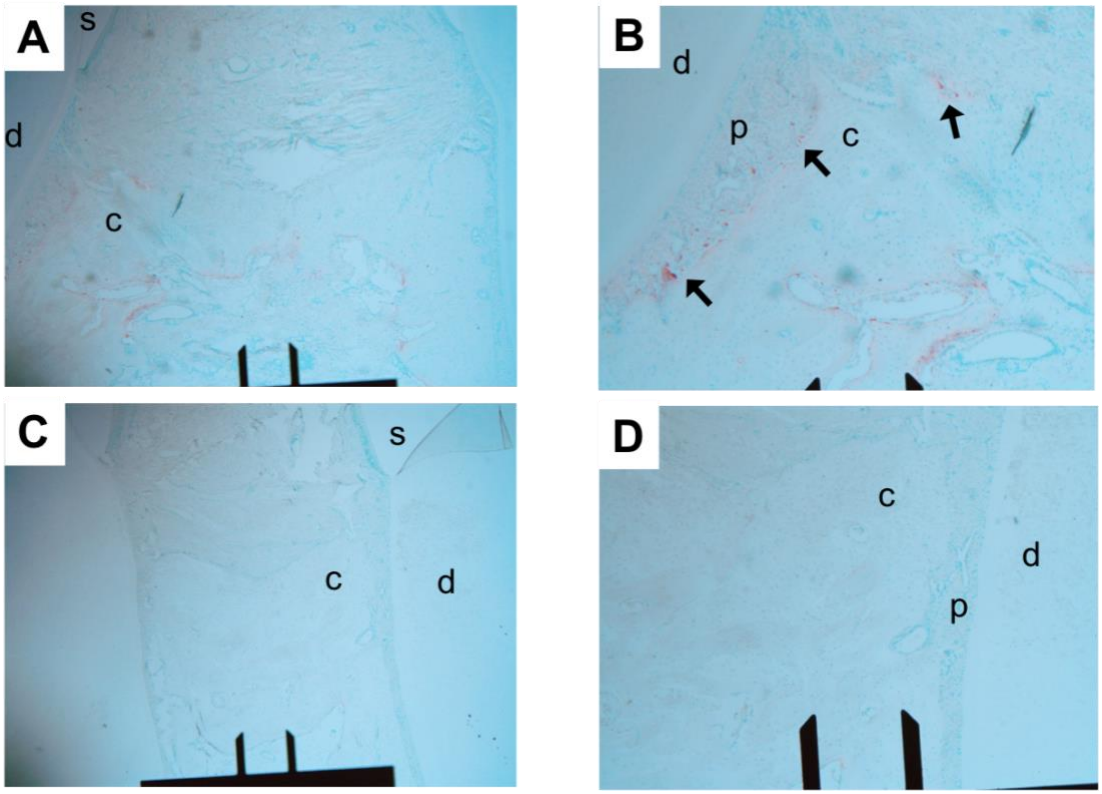

Figure 11. TRAP: Osteoclastic activity 2 weeks after CSF. (A) CSF, 2.5X magnification (B) CSF, 5X magnification (C) Control, 2.5X magnification (D) Control, 5X magnification. Note significant osteoclastic activity in the experimental teeth (A, B) on the crest of the alveolar bone and lamina dura. C=crest, d=dentin, p=periodontal ligament, s=sulcus, arrows indicate TRAP activity and osteoclasts.

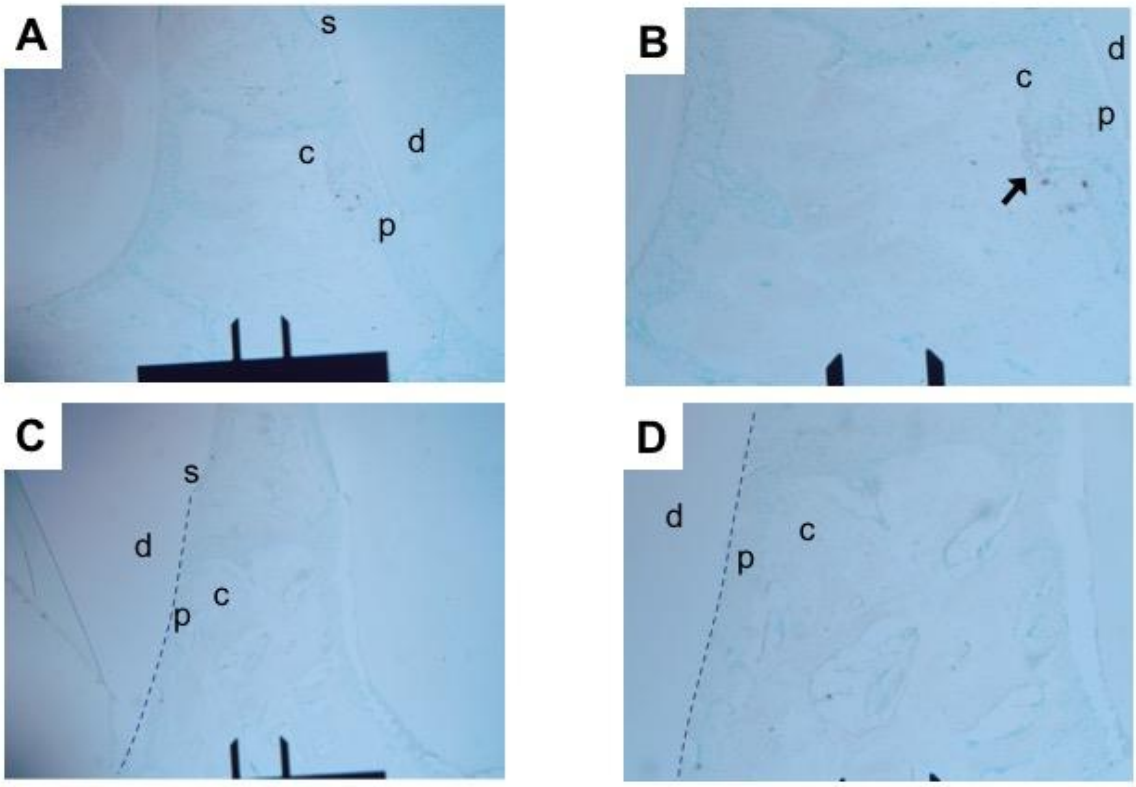

Figure 12. TRAP: Osteoclastic activity 4 weeks after CSF. (A) CSF, 2.5X magnification (B) CSF, 5X magnification (C) Control, 2.5X magnification (D) Control, 5X magnification. After 4 weeks of healing, TRAP activity has decreased to control levels. C=alveolar crest, d=dentin, p=periodontal ligament, s=sulcus, arrows indicate TRAP activity and osteoclast. Dotted line indicates where attachment to tooth has torn during histological preparation.

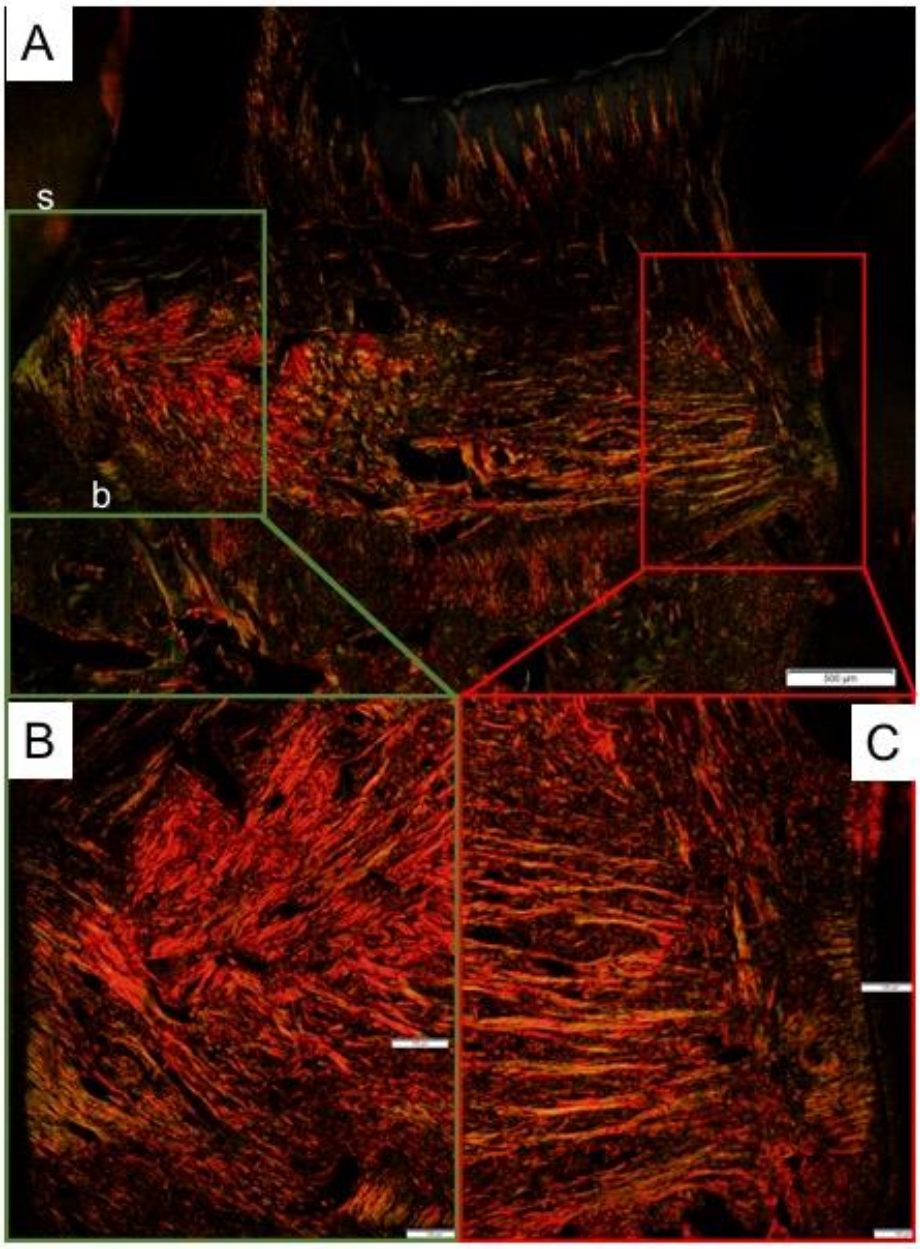

Figure 13. Picro-sirius red: Supracrestal fibers 2 weeks after healing. (A) 2X magnification, (B) CSF, 10X magnification, (C) Control, 10X magnification. Note disorganization of the collagen fibers on the CSF side after 2 weeks of healing. The control side shows distinct, well-organized collagen fibers. b=bone, s=sulcus.

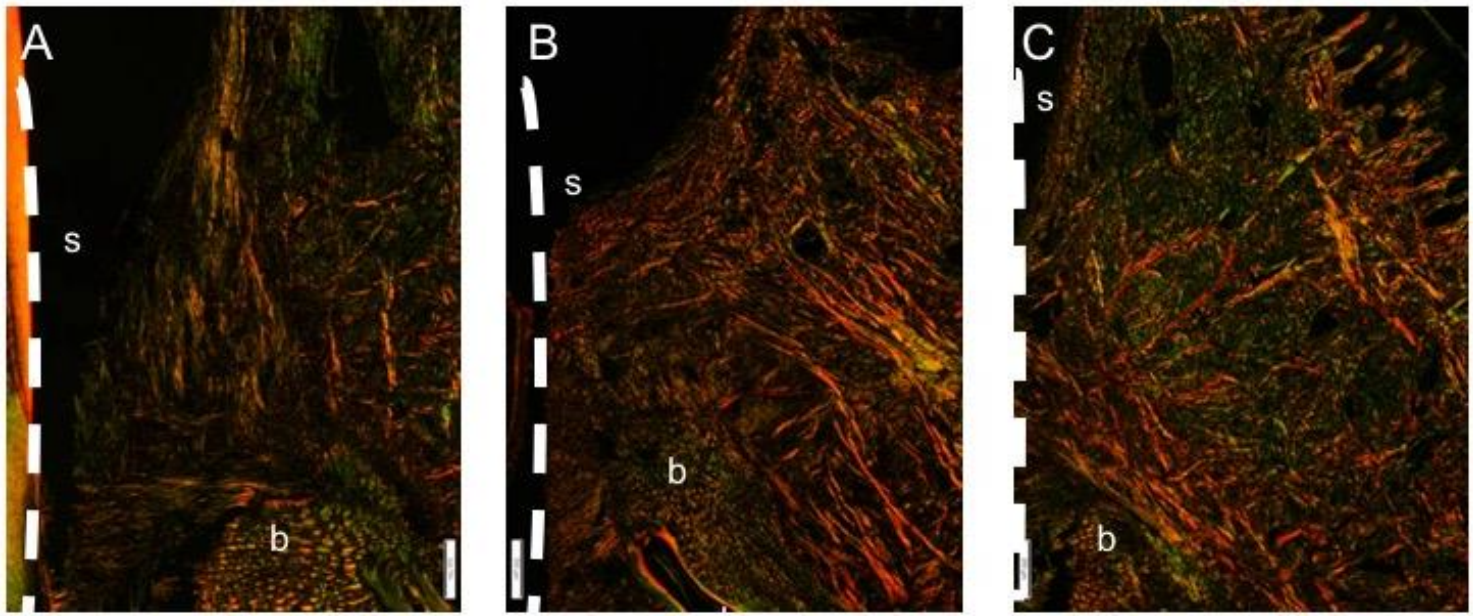

Figure 14. Picro-sirius red: Comparison of supracrestal fibers at 2 weeks and 4 weeks after CSF. 4X magnification. (A) CSF, 2 weeks (B) CSF, 4 weeks (C) Control, 2 weeks. After four weeks of healing, the supracrestal fibers are still disorganized. There does not appear to be a difference in the organization after two weeks and four weeks of healing. Dotted line=root surface, b=bone, s=gingival sulcus