EVALUATION OF MINI-SCREW IMPLANT STABILITY WITH OSTEOCRETE BONE CEMENT

A Thesis

by

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ABSTRACT

The purpose of this study was to evaluate the stability of mini-screw implants (MSIs) placed with and without OsteoCrete bone cement. Using a randomized split-mouth design in 6 skeletally mature male beagle dogs, 23 MSIs were placed in the maxillary alveolar bone with OsteoCrete and 23 MSIs were placed without OsteoCrete. MSI stability was evaluated each week with an Osstell IDx. Histology was used to analyze the surrounding bone of the control and OsteoCrete MSIs. Micro-CT was used to analyze the bone volume fraction and the bone mineral density around the MSIs. The success rate of the control MSIs (87%) was higher than the success rate of the experimental MSIs (78.3%), but the difference was not statistically significant ($p=0.437$). All of the MSI failures occurred between weeks 1 and 4. MSIs inserted with OsteoCrete exhibited an initial decrease in primary stability, but they did not show the increases in secondary stability seen in the control MSIs. Bone mineralization occurred around the outer boundaries of OsteoCrete but not within the OsteoCrete. The OsteoCrete was not being actively remodeled by osteoclasts. There was no difference in bone volume fraction or bone mineral density in the layer of bone around the OsteoCrete and control MSIs. In conclusion, OsteoCrete does not make MSIs more stable; it makes them less stable.

DEDICATION

I would like to dedicate this thesis to my family, especially my parents, Jan and David Hodges, for helping support me financially and emotionally through college, dental school, and orthodontic residency. I would also like to dedicate this thesis to my fiancée, Kathryn Watson, for supporting me during the many hours of working on my research and thesis. Thank you to my twin, Austin Hodges, for being a great research partner and helping with the dog surgeries and analyses.

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All other work conducted for the thesis was completed by the student independently.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

OVERVIEW

Many orthodontic treatments require skeletal anchorage in order to achieve the best results possible. The most commonly used skeletal anchors in orthodontics are mini-screw implants (MSIs). MSIs are important in orthodontic treatment of malocclusions requiring absolute anchorage such as bimaxillary protrusion treatments to obtain maximum retraction, intrusion protocols for open bite treatment, maxillary dentoalveolar distalization in Class II patients, and other orthodontic treatments to prevent unwanted reciprocal forces. MSIs can be placed by the orthodontist; many times, at no additional cost to the patient. Many orthodontists do not consider using MSIs when treating patients due to past experiences with MSI mobility and failures. When MSIs fail it can cause orthodontists to lose confidence in the use of MSIs and it can cause patients to lose confidence in the orthodontist.[1] This can lead orthodontists to provide suboptimal treatment without skeletal anchorage. A meta-analysis and two systematic reviews have reported MSI success rates to be 80%-86.5% in humans.[2-4] The success rates of MSIs are significantly lower than the success rates of titanium endosseous dental implants, which have been reported to be 90% to 100%.[5,6] The field of orthodontics needs a more predictable method for MSI placement, that increases the stability and success of MSIs.

MSIs typically exhibit significant decreases in stability during the first 3 weeks, up to the primary to secondary stability transition.[7] Primary stability, which is

mechanical and due to the cortical and trabecular bone contact with the MSI, decreases due to the osteoclastic removal of bone damaged due to the compression and microfractures during MSI placement. [8-10] Increases in secondary stability are due to cortical and trabecular bone repair and intimate contact with the MSI (osseointegration or bone-to-implant contact). If the primary stability could be enhanced, it would minimize the initial decrease in stability and increase overall stability, leading to more successful MSIs.

Primary stability could be enhanced if there were a bone cement that diffuses into the trabecular bone and bonds to the MSI. Assuming that the cement resorbs over time, this would enhance the initial mechanical stability until full secondary stability could be achieved. OsteoCrete is a magnesium based bone cement that has been shown to increase the stability of stainless steel screws in a horse study.[11] OsteoCrete has been shown to be both biocompatible and osseoconductive.[12,13] A dog study using immediate dental implants showed that there was new bone formation over the top of the OsteoCrete and minimal inflammatory reaction.[13] There have not been any studies that have investigated at MSI placement with adjunctive bone cements.

The purpose of the present study was to evaluate the stability of MSIs placed with and without OsteoCrete bone cement. Another purpose was to evaluate the tissue response to the OsteoCrete.

The hypothesis of the present study was that there would be a significantly higher stability for SLA MSIs placed with OsteoCrete compared to SLA MSIs only.

LITERATURE REVIEW

History of the Use of Implants in Dentistry

Gainsforth and Higley first reported the use of implants for orthodontic anchorage in 1945.[14] They drilled 2.4 mm pilot holes and placed 3.4 mm x 13 mm vitallium screws in the ramus of six mongrel dogs, with the goal of using an elastic from the screws to retract the maxillary canines. All of the vitallium screws failed within 16 days to 29 days after placement. They attributed loosening and failure of the screws to possible cellular reaction to the vitallium screws or the exposure of the screws to the oral cavity and subsequent microorganisms. The first clinical report using an implant for orthodontic anchorage was by Creekmore and Eklund in 1983.[15] They placed a 13 mm vitallium screw below the anterior nasal spine and used it for anchorage to intrude the maxillary incisors to correct the patient's deepbite and excessive gingival display.

Since then, numerous clinical studies using endosseous dental implants for orthodontic anchorage have been published.[16-18] In 1964, Branemark et al found that it was possible to secure a firm anchorage of titanium appliances in the bone. [19] The appliance was very stable with no undesired side effects on the hard or soft tissues. They also reported osseointegration of the titanium implants, defined as "a direct – on the light microscopic level – contact between living bone and implant", which is the most important factor for the success or failure of implants.[20] The use of endosseous dental implants in the palate and retromolar area allowed for absolute orthodontic anchorage. However, there are numerous disadvantages associated with the use of endosseous dental implants for orthodontic anchorage. First, an endosseous dental

implant requires a surgical procedure for placement and removal, which results in significant morbidity and cost. Second, after the placement of these implants there is a two to six month healing period for osseointegration prior to loading the implants. Third, the size of the implant was sometimes larger than the space available for placement.

Kanomi was aware of these limitations and sought out an alternative to the use of endosseous dental implants.[21] He introduced the first mini implant for orthodontic use. The mini screws were 1.2 mm in diameter and 6 mm long, which opened up many more placement sites. The mini implants that Kanomi used were originally designed to be used for fixation with bone plates in craniofacial surgeries. Kanomi stated that "a mini-implant for orthodontic anchorage should be small enough to place in any area of the alveolar bone, even in apical bone. The surgical procedure should be easy enough for an orthodontist or general dentist to perform and minor enough for rapid healing. The implant should be easily removable after orthodontic traction." The question of how long and how wide a mini screw implant needs to be has been well studied and manufactures have produced many different sizes of titanium MSIs. The diameters of most MSI systems range from 1 to 2 mm and the lengths range from 3 to 14 mm.

MSIs are being used by more orthodontists each year. A survey of AAO members in 2008 found that 54.5% of the 564 respondents were placing their own MSIs.[1] A survey in 2011 found that the majority of the orthodontists, 69.2% of private practitioners and 82.9% of residency programs placed their own MSIs.[22] This study also found that practitioners reported success rates of 83.9% for self-drilling MSIs, while the

mean for the residency programs was 80.1%. There have been three studies, two systematic reviews and one meta-analysis, reporting MSI failure rate ranged from 13.5%-20%.[2-4] Failures can result in the patient and parent losing confidence in the orthodontist, extended treatment time, and loss of anchorage. Research continues to be done looking into ways of increasing the stability of MSIs.

Failure and Success

While MSIs continue to be used more and more in academic and private orthodontic practices, their failure rates remain less than desired. In most cases, it is necessary for the MSI to remain stable for at least 6 months and up to two years. The failure rates of MSIs have been reported to vary greatly. However, two systematic reviews and one meta-analysis found the MSIs fail at rates of 13.5% to 20%.[2-4] The success rates of MSIs are significantly lower than the success rates of titanium endosseous implants, which have been reported to have success rates of 90% to 100%.[5,6] In 2015, Moraschini et al performed a systematic review that included 7711 endosseous implants, with a mean follow up time of 13.4 years, and found the success rate was 94.6%.[5] In 2012, Papageorgiou et al performed a meta-analysis of 52 studies, which included 4987 miniscrew implants inserted in 2281 patients.[2] The overall failure rate was 13.5% with a 95% confidence interval of 11.5-15.8%. In 2009, Reynders et al performed a systematic review of 19 articles, with each article reporting on between 12 and 480 miniscrew implants.[3] Success rates were between 0% and 100%, but most articles reported greater than 80%, or failure rates of less than 20%, if displaced and

mobile implants were not considered failures. In 2009, Schatzle et al performed a systematic review of 27 articles, with a total of 2374 miniscrews inserted in 1196 patients. There were 363 MSIs that failed, representing a15.3% failure rate.

The MSI success rate is good enough to advocate the use of MSIs when they are needed for maximum anchorage or poor compliance. However, the orthodontist is going to have to replace an MSI in 13.5% to 20% of the cases due to failure which could result in extended treatment time, patient loss of confidence, increased patient discomfort, and increased cost to the orthodontist. If the stability and success rate of MSIs could be improved it would help decrease all of the problems listed above. In a 2008 survey of AAO members on miniscrew usage performed by Buschang et al, they found that orthodontists with failure rates of greater than 25% were much less satisfied with MSIs when compared to the orthodontists with less than 10% failure rates, who were almost all satisfied with MSIs.[1]

There are a number of hypotheses that have been proposed to explain MSI failures: cervically placed MSI's may have deflection of the alveolar bone, MSI's placed near the periodontal ligament of teeth, low bone density, thin cortical plates, excessive pressure during placement causing trabecular bone microfractures, and excessive gingival thickness causing a decreased amount of MSI within the bone.[23] The interdental alveolar bone is flexible and deformable. The more cervical the interdental alveolar bone the more delicate the bone is. This can result in a decreased primary stability. The bases of the maxillary and mandibular alveolar processes are not as flexible and deformable, thus resulting in a better placement site for successful mini

screws. With these two considerations; the more cervical an MSI is placed the increased risk of failure, compared to the more apical an MSI is placed the better the prognosis.

Bone Healing

The events that occur during bone healing around implants appear to be the same as the events that occur during fracture healing.[24] There are four major stages of bone healing: hematoma, clot resolution, osteogenic cell migration, and de novo bone formation. The first thing that occurs in implant placement and fracture healing is the formation of a hematoma (blood clot). The hematoma is made up predominately of red blood cells and platelets. While the red blood cells serve little purpose in bone healing, the platelets provide a number of growth factors and vasoactive factors through platelet degranulation. These factors help to recruit neutrophils, macrophages, and mesenchymal cells, including fibroblasts and osteoblasts, from the circulation and adjacent bone marrow. Platelet degranulation also helps to activate the cascade to form the fibrin within the blood clot. The osteogenic cells migrate to the implant surface through the fibrin within the hematoma which is aided by the cytokine and growth factor release from the platelets and leukocytes. These osteogenic cells are then available to synthesize de novo bone on the actual implant surface (contact osteogenesis). There are also osteogenic cells which synthesize de novo bone on the existing bone surrounding the implant (distance osteogenesis). This is the process that leads to true bone-to-implant contact (BIC) around the implants. BIC is what is responsible for the sustained stability (secondary stability) and long term success of the implant.[9]

In 2003, Berglundh et al performed a study to evaluate the process of new alveolar bone formation adjacent to dental endosseous implants in dogs.[9] They used 120 endosseous implants in 20 labrador dogs to evaluate bone healing between 2 hours and 12 weeks. After 2 hours, the wound chamber was filled with coagulum, including large numbers of erythrocytes and inflammatory cells. After four days, the wound chamber tissue was rich in vascular structures, connective tissue cells trapped in organic stroma, and some inflammatory cells were present. After one week, the first signs of bone formation were observed, with primary spongiosa including trabeculae and woven bone present around the vascular units with osteoblasts and osteocytes. After two weeks, there was mineralized bone contacting the entire SLA surface. The woven bone contained osteocytes and osteoblasts were lining the trabeculae. After six weeks, there was a mix of woven bone and parallel-fibered lamellar bone. After 8 and 12 weeks, there was mature bone with bone marrow in contact with the SLA surface of the implant. It has been estimated that wound healing, along with bone remodeling, occur about 1.5 times faster in dogs than in humans; so this would put the transition of primary and secondary stability of implant healing in humans at approximately three weeks post-operative.

Primary and Secondary Stability

MSIs stability can be depicted by two curves: the primary stability curve, which pertains to the retention of the MSI produced by the initial contact between the implant and bone, and the secondary stability curve, associated with the remodeling and

deposition of new bone around the implant over time (osseointegration/bone implant contact).[9,25,26] Primary stability occurs right after the implant is placed. On the other hand, secondary stability is more of a biological process that is caused by the osseointegration of the MSI with deposition/remodeling of bone around the implant.[8,9]

MSIs can fail if there is not intimate contact between the cortical bone (i.e. due to the lack of primary stability) and the implant.[27] Failure can occur if the implant moves or is not tightly screwed into the bone.[8] One common mistake during implant placement that can lead to a decrease in primary stability is wobbling, or various lateral forces, during implant placement with a manual driver. MSIs can also fail if the biological process of osseointegration (secondary stability) does not occur. Primary stability has been reported to be critical for the success of implants and the development of secondary stability.[28] Ivanoff et al found that the total initial instability of the implants results in less bone formation around the implants in rabbits.[29] The lower primary stability resulted in less bone to implant contact. Javed et al performed a study evaluating the role of primary stability leading to successful osseointegration of titanium dental implants.[30] They found that the main factors influencing primary stability include bone quantity, bone quality, surgical technique, and various implant designs. The factors that influence secondary stability include primary stability, normal bone remodeling, and various implant surface conditions. The implant must mechanically adapt, after initial placement, to the host bone until secondary stability can be achieved.

Using four male adult *macaca fascicularis* monkeys, Melsen et al evaluated implants used for orthodontic anchorage being immediate loaded.[31] They performed

histomorphometric analyses on the MSI samples and found that the osseointegration ranged from 10% to 58% depending on the amount of time after MSI insertion. Osseointegration was independent of the type of bone and applied force level. Osseointegration increased each month up to the final sample (six months).

Ure et al evaluated the stability of 1.6 mm diameter and 9 mm long MSIs using the Osstell Mentor in a split mouth design. They drilled 1.1 mm diameter and 3 mm deep pilot holes prior to MSI insertion. They found that the stability decreased during the first three weeks and began to increase during the three to five week period. They graphically demonstrated the transition from primary stability, week one through three, to secondary stability, all weeks after three. They also found that the stability values between the pilot hole side and no pilot hole side were comparable.[7]

Methods of Primary and Secondary Stability Measurement

There are a number of methods to assess stability of implants. The two main types of evaluations used are biological and biomechanical. The biological evaluations include: histomorphometric analysis, micro-computed tomography, and radiographic analysis. The biomechanical evaluations include: resonance frequency analysis, insertion torque, removal torque, pullout test, percussion test, cutting torque resistance analysis, and impact hammer method.[32-34]

Histomorphometric Analysis

Histomorphometric analysis has been utilized by numerous studies to evaluate the amount of BIC with orthodontic MSIs and dental implants.[35-37] The

histomorphometric analysis can be performed using decalcified sections without the MSI in the specimen and stained with H&E or embedding the specimens in methyl methacrylate with the MSI present. The two available methods give a two dimensional estimate of BIC and allows for the microscopic study of the cells and tissue.

For example, Woods et al measured BIC of MSIs to determine the effect of force, timing, and location.[36] They utilized histomorphometric analyses using a light microscope and Metamorph software. Their samples were dehydrated using a series of ethanol baths, embedded with methyl methacrylate, and horizontally sectioned with a Buehler Isomet Saw. The 125 micron sections were mounted on glass slides and polished to 70 microns using silicon carbide paper. The sections were stained using Stevenel's blue and then Van Geison counterstain. The sections were imaged at a 2.5x magnification. The percent BIC of each MSI was measured at three different levels: coronal (cortical), middle trabecular, and apical trabecular. They concluded that only limited amounts of osseointegration could result in adequate implant stability as determined by histomorphometric analyses.

Freire et al performed histomoporphometric analyses on their MSI specimens using traditional H&E.[35] The specimens were decalcified by soaking them in EDTA and formic acid for 14 days and 48 hours, respectively. The MSIs were removed from the decalcified specimens. They were then mounted in paraffin and were sectioned at 5 microns down the mesio-distal long axis. The 5 micron sections were stained with H&E for light microscopy evaluation. The specimens were analyzed at 40x magnification.

The BIC in the histomorphometric measurements was determined by measuring the perimeter of bone and implant interaction.

Micro-computed Tomography

Micro-computed tomography (Micro-CT) is an invasive, non-destructive method that can be used to assess bone around implants. Micro-CT is invasive because the sample of interest must be removed from the subject in order to be scanned. It is non-destructive because the sample can be used at a later time for histology or various other measurements. Micro-CT evaluation produces high resolution, three dimensional, images of bone samples that allow quantitative analysis of the cortical and trabecular bone around an implant.[38] Micro-CT allows for 3D quantitative analysis of the bone surrounding the entire implant, whereas histomorphometric analysis only allows for 2D quantitative analysis of limited ground sections of each specimen.[39] Numerous studies have compared the morphometric results of trabecular bone obtained from micro-CT with the histomorphometric results of the same specimens.[39-42]

Butz et al found that the correlation between histology and micro-CT was significant for cortical ($r = 0.65$, $P < .05$) and cancellous bone ($r = 0.92$, $P < .05$) at 24 to 240 microns from the implant surface, but there was no significant correlation found for the 0 to 24 micron region from the implant surface.[40] Their specimens were scanned at 8 micrometers. They stated that there is a need for further research to address the inherent metallic halation artifact, which can confound the bone assessment near implants when using micro-CT.

Rebaudi et al reported that measurements of bone-to-implant contact obtained by micro-CT were similar to those obtained with standard undecalcified histology.[39] They also stated that there was a 45 micrometer thick area surrounding the surface of the implant which could have obscured the bone measurements. They scanned the specimens at 20 micrometers so if a higher resolution scan is performed the metal halation artifact could be reduced.

Van Oosterwyck et al qualitatively compared micro-CT slices and histological sections from the same specimens.[41] They found that the overall trabecular structure is very similar according to both techniques. They also found that the titanium implant does not seem to produce significant artifacts even very close to the interface. However, they did note that there were areas around the threads of the implants on the micro-CT images that could be metal halation artifacts. The specimens were scanned at a resolution of 60 micrometers.

In 2011, Ikeda et al assessed bone volume fraction (bone volume/total volume) in the peri-implant area of SLA and machine surfaced MSIs using micro-CT at a resolution of 6 micrometers.[32] They analyzed the regions from 6 to 24 micrometers and 24 to 42 micrometers and were able to accurately visualize and quantify the bone volume fraction of the cortical and trabecular areas. The 0 to 6 micrometer area was not evaluated because of the potential metallic halation effects. Massey et al also used this technique for assessing the bone volume fraction around MSIs that had been loaded with either 200 or 600 g of force.[43] They evaluated three layers of bone extending 6 to 24 micrometers, 24 to 42 micrometers, and 42 to 60 micrometers from the surface of the MSI.

Resonance Frequency Analysis (Osstell Mentor)

Resonance frequency analysis (RFA) is a non-invasive method that quantitatively determines implant stability.[28,44-46] Current RFA systems are considered third generation and are wireless, battery powered, and portable.[47] The measurements are given as implant stability quotients (ISQ) which are valid for the transducer that is calibrated for each specific type of implant by the manufacturer. This allows the comparison of ISQ values with different types of implants.

The Osstell Mentor system is a third generation RFA system, consisting of a small metal peg transducer (smartpeg) that screws into the head of the MSI. The smartpeg contains a magnet at the top of the peg that is excited by the handheld wand that emits magnetic pulses. This results in micro-vibrations of the smartpeg that the computer quantifies as the implant stability quotient (ISQ). The ISQ can range from 1 (lowest stability) to 100 (highest stability). Higher ISQ values indicate increased stability, whereas lower ISQ values indicate decreased stability.[48] The ISQ value depends on three factors: the transducer design, the stiffness of the implant and its BIC, and the implant length above of the marginal bone level.[47,49]

ISQ quantifies the MSI stability by providing an indirect measurement of osseointegration. Ersanli et al used RFA to evaluate the stability of dental implants during the osseointegration period. They found that the ISQ values decreased during the first three weeks after placement and recovered back to near initial ISQ values at the loading appointment (3 or 6 months).[50]

Huang et al performed a finite element model study using RFA to assess the differences of implant stability in different bone qualities.[46] They found that the RFA values were almost four times higher in type I bone than in type IV bone. They also found that as density decreases in type III bone RFA values decrease in a linear fashion.

OsteoCrete

If primary stability could be enhanced, then the primary stability curve would not decrease or decrease more slowly. This would enhance overall stability two ways. First, it would ensure that MSIs remain more stable during the first 3 weeks, allowing healing and secondary stability to proceed without a dip in the stability curve. Second, it would enhance overall stability because secondary stability is related to primary stability (see review above). Primary stability could be enhanced by bone cement that maintains the stability of MSIs.

OsteoCrete is a magnesium phosphate biomaterial that is osteoconductive, biohesive, and has high strength characteristics.[11,12,51,52] OsteoCrete currently has FDA 510(k) approval as a bone void filler for pelvis and long bone applications. The formulation of OsteoCrete allows it to be both injectable and moldable. OsteoCrete has two components that are mixed together to form magnesium potassium phosphate. One component is the granular, powder portion containing monopotassium phosphate (54%), magnesium oxide (33%), small amounts of tricalcium phosphate (9%), and dextrose (4%); the other component is a modified saline solution.[11] The components are mixed in a plastic mixing bowl with a mixing spatula. The sterile saline solution is poured into

the bowl first; then the granular portion is poured in. The mixture is stirred vigorously for 2 minutes. After the mixing stage it remains in a fluid, injectable state for the first 3 minutes and, if allowed to set, it becomes moldable during the subsequent 3-5 minute interval. The mixture reaches complete set after approximately 10-15 minutes. OsteoCrete exhibits a slight amount of expansion upon setting, which may be beneficial for retention. OsteoCrete has been shown to resorb approximately 50% after 24 weeks in rabbits.[12]

An in vivo study comparing different bone cements showed positive results for the use of OsteoCrete. [11] They evaluated the bone-screw interfaces of screws inserted into the third metacarpal and metatarsal bones of horses. The study compared stainless steel (SS) bone screws (these were the controls), SS bone screws with Ca-cement, SS bone screws with Mg-cement (OsteoCrete), and SS bone screws with polymethylmethacrylate (PMMA). The peak removal torque values for each group were 1,701 +/- 164 Nmm, 1,665 +/- 148 Nmm, 2,383 +/- 198 Nmm, and 1,981 +/- 240 Nmm, respectively. These differences were statistically significant, demonstrating a 40% increase in peak torque to failure in the OsteoCrete group compared to the controls. This study measured the peak torque to failure rather than the pullout strength. They stated that the pullout test evaluates the biomechanical holding power of the screw and the material surrounding it. The peak removal torque value more closely reflects the strength and bonding characteristics of the screw interface as well as the resistance of the screw to back out from cyclic forces. This is the most common mechanism of failure observed in situations clinically.

In 2013, Sehlke et al performed a study to determine whether OsteoCrete can be used to stabilize dental implants in large extraction sockets and whether it is replaced by native bone.[13] They extracted the mandibular third premolars and first molars of four mongrel dogs and placed immediate dental implants; the control sites received dental implants only and the experimental sites received OsteoCrete and dental implants. All four dogs were euthanized four months after implant placement. They performed histology and electron microscopy to analyze the BIC and the OsteoCrete surface texture and porosity. Two of the experimental implants failed and the BIC of one of the control implants could not be calculated due to complete soft tissue integration. They performed transmucosal single stage implant and abutment placement on the first dog. However, after initial healing it was observed that the OsteoCrete had high levels of bacteria and plaque on the surface when exposed to the oral cavity. This caused the OsteoCrete to discolor, soften, and dissolve. They modified the study protocol and attempted primary closure in dogs 2, 3, and 4.

All previous studies of OsteoCrete were performed under a closed, sterile surgical environment.[11-13,51] Upon histologic evaluation, the residual OsteoCrete was inert and did not result in an inflammatory response. There was new bone growth on the top of the OsteoCrete in experimental sites that achieved primary closure. The histologic BIC was 51.7% ± 13.7% for experimental implants and 43.7% ± 8.1% for control implants. The difference in BIC was not statistically significant. Using SEM, they found that the external surface of the OsteoCrete had no external porosities, however it did show microstructural craze lines or cracks which is likely to have

occurred during setting of the material. The authors recommend further investigation of OsteoCrete for dental applications.

No studies have been performed evaluating the effects of OsteoCrete on MSIs inserted into alveolar bone or OsteoCrete being used with SLA surface treated MSIs.

SLA Surface Treatment

Sand blasted large grit acid etched (SLA) implants undergo a subtractive process to increase their surface area. Numerous studies have shown that SLA implants are superior to machined implants, based on removal torque, RFA, histomorphometric, and micro-CT analyses.[9,26,32,53-57] SLA surfaces have been shown to have greater microtopography (increased surface area), which increases fibrinogen absorption.[58] This could explain the observed increase in platelet adhesion.

Buser et al performed a study in 1999 looking at differences in removal torque with machined, SLA, and TPS (titanium plasma sprayed) dental implants in the maxilla of miniature pigs.[54] The machined surface implants had mean removal torque values (RTV) ranging from 0.13 to 0.26 Nm, and the RTV of the SLA and TPS groups ranged from 1.14 and 1.56 Nm. 4 weeks after healing, the SLA implants had higher mean RTV compared to the TPS implants (1.39 vs. 1.14 Nm), but these differences were not statistically significant. They concluded that the removal torque or interface shear strength of titanium implants was significantly influenced by their surface characteristics. This conclusion was based on the fact that the machined titanium surface demonstrated significantly lower RTV when compared with the TPS and SLA

surfaces. Buser et al also performed a histomorphometric study investigating the difference in osseointegration of machined and SLA dental implants.[55] They found that machined surfaces had 20-25% bone-to-implant contact and SLA surfaces had 50-60% bone-to-implant contact when observed on undecalcified transverse histologic sections.

Klokkevold et al performed a study in 1997 using acid etched and machined implants in the femurs of ten New Zealand White rabbits.[53] They found the removal torque value was 4 x higher for the acid etched surface implants when compared to the machined surface implants. The mean removal torque values were 20.50 +/- 6.59 Ncm and 4.95 +/- 1.61 Ncm for the acid etched and machined surfaces respectively.

Pilot Holes

A recent study using the Osstell Mentor to evaluate stability and the bone surrounding the MSIs found that pilot holes increase the initial stability, but they significantly decrease the stability over time. It was thought that the pilot hole caused damage to the surrounding bone due to the drilling process and most likely overheating of the bone.[33] This is the reason the present study used "down-sized" MSIs with a hand-driver to place the pilot holes. This should reduce the confounding variable of thermal bone damage which can be caused by the use of a pilot hole drill. There is not a way to deliver the OsteoCrete into the bone prior to MSI placement without placing a pilot hole.

CHAPTER II

EVALUATION OF MINI-SCREW IMPLANT STABILITY WITH OSTEOCRETE BONE CEMENT

INTRODUCTION

Many orthodontic treatments require skeletal anchorage in order to achieve the best results possible. The most commonly used skeletal anchors in orthodontics are mini-screw implants (MSIs). MSIs are important in orthodontic treatment of malocclusions requiring absolute anchorage such as bimaxillary protrusion treatments to obtain maximum retraction, intrusion protocols for open bite treatment, maxillary dentoalveolar distalization in Class II patients, and other orthodontic treatments to prevent unwanted reciprocal forces. MSIs can be placed by the orthodontist; many times, at no additional cost to the patient. Many orthodontists do not consider using MSIs when treating patients due to past experiences with MSI mobility and failures. When MSIs fail it can cause orthodontists to lose confidence in the use of MSIs and it can cause patients to lose confidence in the orthodontist.[1] This can lead orthodontists to provide suboptimal treatment without skeletal anchorage. A meta-analysis and two systematic reviews have reported MSI success rates to be 80%-86.5% in humans.[2-4] The success rates of MSIs are significantly lower than the success rates of titanium endosseous dental implants, which have been reported to be 90% to 100%.[5,6] The field of orthodontics needs a more predictable method for MSI placement, that increases the stability and success of MSIs.

MSIs typically exhibit significant decreases in stability during the first 3 weeks, up to the primary to secondary stability transition.[7] Primary stability, which is mechanical and due to the cortical and trabecular bone contact with the MSI, decreases due to the osteoclastic removal of bone damaged due to the compression and microfractures during MSI placement. [8-10] Increases in secondary stability are due to cortical and trabecular bone repair and intimate contact with the MSI (osseointegration or bone-to-implant contact). If the primary stability could be enhanced, it would minimize the initial decrease in stability and increase overall stability, leading to more successful MSIs.

Primary stability could be enhanced if there were a bone cement that diffuses into the trabecular bone and bonds to the MSI. Assuming that the cement resorbs over time, this would enhance the initial mechanical stability until full secondary stability could be achieved. OsteoCrete is a magnesium based bone cement that has been shown to increase the stability of stainless steel screws in a horse study.[11] OsteoCrete has been shown to be both biocompatible and osseoconductive.[12,13] A dog study using immediate dental implants showed that there was new bone formation over the top of the OsteoCrete and minimal inflammatory reaction.[13] There have not been any studies that have investigated at MSI placement with adjunctive bone cements.

The purpose of the present study was to evaluate the stability of MSIs placed with and without OsteoCrete bone cement. Another purpose was to evaluate the tissue response to the OsteoCrete.

The hypothesis of the present study was that there would be a significantly higher stability for SLA MSIs placed with OsteoCrete compared to SLA MSIs only.

MATERIALS AND METHODS

Six periodontally healthy, beagle dogs 1 to 2 years of age, weighing between twenty and twenty-five pounds, served as the experimental models. The dogs were purchased from Marshall Bioresources (DBA Marshall Farm Group, North Rose, NY). The project was approved by the Institutional Animal Care and Use Committee at Texas A&M University College of Dentistry (IACUC #2015-0294-CD).

The animals were quarantined for 10 days. On the day of OsteoCrete (Bone Solutions Inc, Colleyville, TX, USA) and mini-screw implant (MSI) placement, they were placed under general anesthesia using IM sedation, consisting of a combination of ketamine (2.2 mg/kg) and xylazine (0.22 mg/kg) and intubation, followed by 1% to 2.0% isoflurane in oxygen at 0.5 to 1 L per minute. Vital signs were monitored throughout each procedure. All dogs received a prophylaxis using an ultrasonic cavitron (Denstply, York, PA), irrigated with a 0.12% chlorhexidine gluconate solution. A Planmeca Intra-Oral X-Ray unit (Planmeca USA, Roselle, IL) was used to take periapical radiographs of the left and right maxillary quadrants using size 4 phosphorous films. The radiographs were used to determine ideal MSI placement with adequate inter-radicular space. Local anesthetic, consisting of 2% lidocaine with 1:100,000 epinephrine (Patterson Dental, St. Paul, MN), was administered via local infiltration of the maxillary vestibule with a 27 gauge needle.

The MSIs used were made of medical grade titanium alloy. They were sand blasted large grit acid etched (SLA) surface treated. They were 7 mm long, with a 1.6 mm outer diameter, a 1.1 mm internal diameter, and a 0.7-mm pitch (Neodent, Curitiba, Parana, Brazil) (Figure 1). The head of the screw had a 1 mm high collar and a 1.1 mm internal thread fabricated to accept the Osstell IDx Smartpeg type A3 (Integration Diagnostics, Goteborg, Sweden). The Smartpeg was used to measure the weekly implant stability quotient (Figure 1). The Osstell IDx was calibrated prior to measurements. The Osstell data was represented as the implant stability quotient (ISQ), which ranges from 1 to 100. A larger ISQ indicates increased stiffness or stability.

The experimental and control sides of each dog was randomly allocated using an Excel random number generator. The protocol on the experimental side was to first drill all of the pilot holes in one maxillary quadrant. Immediately after drilling, one package of OsteoCrete was hand mixed for 90 seconds (Figure 2A). The OsteoCrete was then loaded into a 3 cc syringe with a thin-walled 18 gauge BD needle. The needle was sectioned prior to surgery with a dental handpiece and metal cutting bur so that it was blunt and only 7 mm long. The needle was inserted approximately 3 to 4 mm into each pilot hole. Approximately 0.1 mL of OsteoCrete was injected into each of the four pilot holes using positive thumb pressure for approximately 2 seconds. The syringe was backed out as the OsteoCrete was dispensed into the pilot hole (Figure 2B). The lumen of the needle was kept patent with an 0.016” round stainless steel orthodontic wire. The same MSIs used to drill the pilot holes were then placed into the four OsteoCrete filled pilot holes using a hand driver. Each was inserted until the collar of the MSI was at the

level of the gingiva (Figure 2C). A 2x2 gauze was used to wipe away any OsteoCrete and blood that extruded from the site.

The protocol for the control sites in the other quadrant was to hand drill all of the pilot holes using the same 7 mm long SLA surface treated MSIs. They were inserted until the MSI collar was at the level of the gingiva, then removed and replaced to replicate the protocol on the experimental side, albeit without OsteoCrete. Periapical radiographs were taken to ensure proper MSI positioning and to verify that the MSIs had not contacted the roots (Figure 3).

All of the MSIs were placed approximately 2 mm apical to the mucogingival junction in nonkeratinized gingiva. There were four MSIs placed in each buccal quadrant of the maxilla; one dog had only three MSIs placed in each quadrant due to insufficient space. MSI's were placed between the canine and first premolar, and in the inter-radicular spaces of the 2^{nd}, 3^{rd}, and 4^{th} premolars (Figure 4A). All MSIs were placed parallel to the occlusal plane and perpendicular to the cortical plate. There was a total of 46 MSIs placed in the six beagle dogs. Analgesics (Nalbuphene, 1-2mg/kg subcutaneous BID for 3 days then PRN) and antibiotics (Penicillin G procaine with Benzathine, 20,000-40,000 units/kg at surgery) were administered in all cases.

Immediately after placement, the MSIs were stabilized with a hemostat and the Smartpeg type A3 was screwed onto the MSI head and tightened using a thumb and index finger (Figure 4A and 4B). The Osstell IDx transducer was oriented perpendicular to the long axis of the MSI and three separate measurements were recorded for each MSI (Figure 4C). The three measurements were averaged. The Smartpeg was removed by

reversing the insertion method. Weekly measurements were taken, resulting in 10 implant stability quotient measurements for each MSI over the nine week experimental period.

The dogs were sedated with 1.1 mL per kilogram of ketamine and 0.1 mg per kilogram of xylazine for the weekly measurements. There was gingival overgrowth around most of the MSIs which inhibited the Smartpeg from being screwed into the head of the MSIs (Figure 4D). The overgrowth was removed using a Vetroson V-10 Bi-Polar Electrosurgical Unit (Summit Hill Laboratories, Navesink, NJ) with the round loop tip. Calcein, alizarin, and calcein were administered at 4 weeks, 6 weeks, and 8 weeks respectively. Calcein (7 mL for 14 kg dog, 10mg/kg) and Alizarin (28 mL for 14 kg dog, 20 mg/kg) were administered intravenously.

After the final measurements were taken at nine weeks, the animals were euthanized with 2 mL of Beuthanasia-D given intravenously and perfused with 1 to 2 L of normal saline solution, followed by 1 L of 4% paraformaldehyde (PFA). The maxillae were resected *en bloc* with a Stryker bone saw and stored in 4% PFA and refrigerated for approximately 3 to 4 weeks. Each of the MSIs and the surrounding bone were trephined parallel to the long axis of the MSIs using a dremel with a 10 mm circular trephine bit (Figure 5A). The maxillae were secured on a survey table that was attached to the platform stand of the dremel to ensure accurate long axis trephining. The dremel was used at approximately 70% max speed, with water irrigation. The specimens were then stored individually in labelled vials with 4% PFA and refrigerated.

Post Mortem Evaluations

Histological Evaluation

Twenty-one specimens were used for fluorescent histology (10 experimental and 11 control) and six specimens were used for H&E histology (3 experimental and 3 control). The specimens were randomly selected.

Confocal fluorescence was used to study the amount and location of OsteoCrete dispersion and new bone mineralization. The fluorescent specimens were dehydrated in graded ethanol for two weeks and soaked in acetone for two days, methyl methacrylate monomer #1 for three days, and methyl methacrylate monomer #2 for four days. They were embedded in methyl methacrylate until polymerization was complete. The samples were sectioned at a low speed, perpendicular to the long axis of the MSIs with a Buehler Isomet Saw (Buehler, Houston, TX) using a diamond wafering blade. The sections, which were approximately 150 μm thick, were ground and polished to approximately 100 μm. Three sections were selected from each specimen to evaluate the cortical, medullary, and apical levels of the MSI and bone. The fluorescent signal was imaged using a Photometrics CoolSnap K4 CCD camera (Roper Scientific, Duluth, Ga) mounted on a fluorescent microscope (Nikon, Melville, NY) and NIS-Elements software (Nikon) at 5x magnification. The fluorescent images for calcein (green) were captured using an excitation of 488 nm and an emission filter between 480 and 600 nm; the fluorescent images for alizarin (red) were captured using an excitation of 561 nm and an emission filter between 530 and 680 nm.

The fluorescent slides were then stained with Stevenel's Blue and Van Gieson and imaged using a Kodak SPOT digital camera mounted on a Zeiss Axioplan microscope (Carl Zeiss Microimaging, Germany) and SPOT 5.0 Software (SPOT Imaging Solutions, Sterling Heights, MI) at 2.5x magnification. Each slide was imaged on automatic contrast and then on higher intensity light in order to visualize the MSI and OsteoCrete separately.

The H&E histology allowed visualization of inflammatory, osteoblast, and osteoclast activity. The six specimens were decalcified in ethylenediaminetetraacetic acid (EDTA) for approximately three months (the MSIs were removed after 1 month of decalcification), dehydrated in graded ethanol, cleared with xylene, and embedded in paraffin. The blocks were then sectioned with a microtome in a perpendicular direction to what was the long axis of the MSI at a thickness of 6 μm. The sections were mounted on glass slides, deparaffinized, rehydrated, and stained with hematoxylin and eosin. These samples were also imaged using a Kodak SPOT digital camera mounted on a Zeiss Axioplan microscope (Carl Zeiss Microimaging, Germany) and SPOT 5.0 Software (SPOT Imaging Solutions, Sterling Heights, MI).

MicroCT Evaluations

Fifteen MSIs from each of the experimental and control groups were randomly selected for μCT analysis (Bruker's Skyscan 1173). Two specimens, along with 4% PFA, were placed one on top of the other in a 14 mm diameter micro CT holder. The specimens were scanned using a high isotropic resolution of 10 μm. X-ray energy levels were set to 130 kVp, current was set to 61 uA, and integration time was 1000ms. A

0.25mm Brass filter and a high resolution setting of 958 projections per 180º were used. The volume of interest of each specimen was defined as a cylinder (6000um diameter) with the implant positioned in the center (Figure 6). The average scanning time was 48 minutes per specimen. Based on ten randomly chosen specimens, grayscales of 45-255 were used as thresholds to represent bone and the MSIs. The grayscales of 110-255 were used to represent only bone, removing the titanium screws so that they were not included in the analysis. Datasets were reconstructed with Skyscan Nrecon software (Bruker, Kontich, Belgium). Reconstruction settings included a Gaussian smoothing of 2, Ring Artifact Correction of 5, Beam Hardening Correction of 20%, and Dynamic Range of [-0.003-0.05].

The region of interest (ROI) was defined as 250 µm apical to the buccal cortical bone and extending 3 mm apically from that. This was determined using the reconstructed 3-dimensional images and evaluating the length of the MSI within the alveolar bone prior to reaching the sinus. All MSIs were within at least 3 mm of bone along their long axis from buccal cortical bone to maxillary sinus cortical bone. The calculated ROI included a 10 µm layer of bone that extended 10 to 20 µm from the MSI surface (Figure 7). The voxel of bone adjacent to the MSI surface (0-10 µm) was excluded because it was subject to metallic halation artifacts. Bone volume fractions (bone volume/total volume) and bone mineral density (g/cm^3) were calculated for each ROI. 3-D renderings were made using the Nrecon software (Bruker, Kontich, Belgium) to show the density and trabeculation of the bone around the MSIs.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 22.0 Software (SPSS Inc.; Chicago, IL) was used for the statistical analyses. The Osstell IDx Implant Stability Quotients (ISQs) were normally distributed. Means and standard deviations were used to describe the data and analyzed using paired samples t-tests. The success and failure rates were analyzed using a chi-square test. Bone volume fraction (TV/BV) and bone mineral density, which were not normally distributed, were described using medians and interquartile ranges. They were analyzed using Wilcoxon Signed Ranks Tests.

RESULTS

Success Rates

There were three MSIs that failed on the control side and five that failed on the experimental OsteoCrete side (Table 1). The MSIs were considered unsuccessful if they fell out between measurements or if they were mobile enough to where they were removed during placement and removal of the Smartpeg. Only 2 of the MSIs fell out between measurements. The remainder of the unsuccessful MSIs were mobile enough that they pulled out during careful placement and removal of the Smartpeg. This resulted in a success rate of 87% (20 of 23 MSIs) for the control group and 78.3% (18 of 23 MSIs) for the experimental group (Figure 8). This difference was not statistically significant, with a chi-square of 0.6053 and a p-value of 0.437 (Table 2). The tissues were inflamed around all MSIs within one to two weeks, and remained inflamed throughout the nine week experimental period. This required the use of electrosurgery

to remove excessive gingival tissue over the MSIs when placing the Smartpeg for ISQ measurements.

Osstell IDx ISQ

The ISQ values showed an initial decrease in stability during the first two weeks for both the experimental and control MSIs (Table 3, Figure 9). Compared to the experimental MSIs, the control MSIs decreased slightly more from week 0 to week 1, and decreased slightly less from week 1 to week 2. None of the group differences during the first two weeks were statistically significant. The stability of the control MSIs increased markedly between weeks 2 and 5 and more slowly from week 5 to week 9. The experimental MSIs showed only slight increases of stability between weeks 2 and 9. Stability of the control MSIs was significantly higher than the stability of the experimental MSIs at week 4 ($p=0.023$) and at week 5 ($p=0.010$). There also were differences at weeks 6 to 9 that closely approached statistically significant levels (week 6: $p=0.059$, week 7: $p=0.070$, week 8: $p=0.055$, week 9: $p=0.187$).

Confocal Fluorescence

On the fluorescent histological sections the OsteoCrete exhibited a black granulated appearance (Figure 10). OsteoCrete was present in most of the mid trabecular and apical trabecular sections. OsteoCrete was generally not present in the cortical sections. There was no bone or indication of bone mineralization around the SLA MSIs where OsteoCrete was present. There was bone mineralization around the

SLA MSIs where there was no OsteoCrete present. There was no mineralization within the area covered by OsteoCrete, but there was mineralization along the interface between OsteoCrete and bone. The control sections showed intimate bone contact and mineralization around the SLA MSIs. The control sections consistently showed higher bone-to-implant contact than the experimental sections.

Stevenel's Blue

The Stevenel's Blue stained sections showed OsteoCrete around the MSIs as a dark grey area with a granulated appearance (Figure 11). Osteoblasts were observed lining the trabecular bone on the side opposite from the OsteoCrete filled spaces. There were no layers of osteoblasts observed on the side of the bone touching the OsteoCrete (Figure 11 and 12). The bone surface next to the OsteoCrete appeared jagged and shredded. In the control sections, there were layers of osteoblasts observed near the bone around the MSIs and along the trabecular spaces. There were very few osteoclasts observed in the control sections and the OsteoCrete sections. There were no osteoclasts observed against the OsteoCrete. There were osteocytes observed in their lacunae throughout the cortical and trabecular bone of both experimental and control sections.

Hematoxylin and Eosin

One of the experimental sections stained with H&E showed a fairly large area of OsteoCrete that ballooned out on one side of the MSI hole (Figure 13). All of the sections showed areas of OsteoCrete that had a granular appearance, with bone directly

next to it, osteocytes within their lacunae, osteoblasts lining the bone facing away from the OsteoCrete, and no osteoclasts near or around the OsteoCrete. The control sections showed normal bone with osteocytes, osteoblasts along the trabecular bone, and very few osteoclasts. The control sections had trabecular bone around the MSI hole, showing where the MSI had been prior to removal for demineralization. The OsteoCrete sections also showed a circular pattern, but there was less osteoid and bone matrix around the MSI hole.

Micro Computed Tomography

The 3D renderings of the micro CT scans showed that the most apical portions of the MSIs were within the maxillary sinus (Figure 6). The median for bone volume fraction (Bone Volume / Total Volume) was 46.45 for the control group and 48.36 for the experimental group (Figure 14). The median for bone mineral density was 0.70 g/cm^3 for the control group and 0.72 g/cm^3 for the experimental group (Figure 14).

DISCUSSION

MSIs placed into bone along with OsteoCrete are slightly less successful than MSIs placed in bone without OsteoCrete. The success rate of the control MSIs (87%) was within the range of success rates found in the literature, while the success rate of the experimental MSIs (78.3%) was lower than previously reported studies. The chi-square test showed that the failure rates were not significantly different. Another dog study performed by Sehlke et al, found 100% implant survival for immediate placed control

implants and 75% survival for experimental immediate placed implants with OsteoCrete.[13] Various other dog studies have reported similar or slightly higher MSI success rates.[33,34,59] All of the MSIs in the present study were placed within 2 mm of the mucogingival junction in nonkeratinized gingiva. This was necessary due to the limited inter-radicular space available for MSI placement and the short keratinized gingival areas in the beagle dogs. Placement in the non-keratinized tissue could explain why the success rates were somewhat lower. The absence of keratinized mucosa around MSIs significantly increases the risk of infection and failure.[60] Human studies have reported slightly lower success rates (80-86.5%) than dog studies, due to their inability to control confounding factors such as diet, hygiene, trauma, etc.[2-4]

MSIs are most susceptible to failure during the first four weeks after insertion. All of the MSI failures occurred between week 1 and week 4. Ure et al found that there was a significantly greater decreases in stability during the initial 3 weeks after placement for MSIs that failed when compared to MSIs that remained stable.[7] This is when MSIs and dental implants transition from primary stability to secondary stability, when overall stability dips to its lowest level.[7,8,34,61] Berglundh et al found osteoclasts around implants as early as 4 days, new bone as early as 2 weeks, and lamellar bone by 4 weeks.[9] This supports the overall stability curve being least stable around 2 weeks and increasing in stability as bone formation continues and matures.

The control MSIs showed the expected initial loss of primary stability followed by increased secondary stability. Previous studies have shown similar stability curves.[7,32-34] The ISQ data showed a decrease in stability for the control and

experimental groups during the first 2 weeks. The primary stability, which has also been termed mechanical stability, is due primarily to the cortical bone.[8,30] The initial decline in primary stability is due to the remodeling of the cortical bone, with osteoclasts being present as early as 4 days post-implant placement.[8,9] The secondary stability occurs as a result of bone remodeling and healing, with more new bone formation than bone resorption within the cortical and trabecular bone.[8,9,30] This was observed in the fluorescent histology sections with the green, red, green fluorescent labels showing active bone remodeling directly next to the MSI and throughout the adjacent cortical and trabecular bone in control MSIs.

MSIs inserted with OsteoCrete also undergo the initial decrease in primary stability, but they do not show the expected increases in secondary stability. The primary stability decrease occurred similarly in both the control and experimental groups, probably because little or no OsteoCrete was present in the cortical layer. There was a more rapid and greater increase of secondary stability for the control group than the experimental group. This difference could have been due to more limited ability for trabecular bone healing around the MSIs in the experimental group. OsteoCrete likely acted as a barrier for new bone formation. Without new bone around the MSIs, micro-motion, expansion, or cracks and craze lines could result in less stable MSIs. Sehlke et al found that the external surface of the OsteoCrete placed with dental implants had no external porosities. However it did show microstructural craze lines or cracks, which likely occurred during setting of the material.[13] In the present study, craze lines could have also occurred during the placement and removal of the Smartpeg during weekly

stability measurements. Native bone has the ability to self-repair, whereas OsteoCrete does not. In contrast to the present results, Hirvinen et al found that stainless steel screws placed with OsteoCrete in horse metacarpal bones had 40% greater removal torque than stainless steel screws placed without OsteoCrete.[11] It is possible that the OsteoCrete cement has different bonding characteristics with stainless steel than with the titanium MSIs used in our study. The OsteoCrete flaked or crumbled off of the titanium MSIs during pilot testing. Cadaveric orthopedic studies have shown that vertebral screws placed with polymethylmethacrylate or calcium phosphate cements also have an increased pullout strength.[62] The polymethylmethacrylate had the highest pullout strength, but it is non-resorbable and would not allow normal healing after the removal of the MSI. Moreover, cadaveric pullout strength does not closely relate to in vivo stability, which limits the clinical applicability of their results.[62]

Bone mineralization and remodeling occur directly next to and around MSIs placed without OsteoCrete. The fluorescent histological images and Stevenel's blue histological images showed bone mineralization occurring directly next to the MSI and throughout the cortical and trabecular bone of the control sections. Numerous other dog studies have reported similar bone mineralization around MSIs.[33,34]

Bone mineralization occurs around the outer boundaries of OsteoCrete but not within the OsteoCrete. Comparison of the same fluorescent and Stevenel's blue histological sections made it possible to differentiate the areas of bone that underwent active bone mineralization. This showed that there was not any bone mineralization occurring within the OsteoCrete. Increased pressure from the injection of the

OsteoCrete could have resulted in the loss of blood supply to these areas, with resultant necrosis of the osteoblasts. Alternatively, it may just act as a physical barrier that inhibits the osteoblasts from laying down new bone. The histological sections showed bone in direct contact with OsteoCrete but there were no osteoblasts, suggesting that OsteoCrete limited new bone formation. The opposite side of the bone (i.e. the side away from the OsteoCrete) appeared normal with a layer of osteoblasts. There was bone mineralization occurring in the areas directly around the OsteoCrete. Selkhe et al reported that the OsteoCrete appeared to be inert and did not result in an increased inflammatory response.[13] They also showed that there was new bone growth over the OsteoCrete in the experimental sites that achieved primary closure. This supports the claim made by OsteoCrete that it is osseoconductive, meaning that it allows bone formation.[11] However, it does not induce bone formation or resorption. In a rabbit study that created cranial defects and monitored bony infill, a narrower gap width and greater bone infill density occurred for the unfilled control sites than the OsteoCrete filled cranial defects.[12] Bone infill progresses quicker when cement does not have to be reabsorbed prior to bone remodeling. This further supports the notion that the OsteoCrete may act as a physical barrier to normal bone formation around the MSI, until it can be either absorbed or resorbed. A rabbit study showed that 50% of the OsteoCrete remained at 24 weeks.[12] Sehlke et al found that their histological specimens showed no adverse biologic response to OsteoCrete, but only minimal replacement at 4 months.[13] The time it takes OsteoCrete to resorb may limit its use in orthodontics.

The OsteoCrete is not being actively remodeled by osteoclasts. The present histological results showed similar numbers of osteoclasts on the experimental and control sections. They also did not show more inflammatory cells around the OsteoCrete, indicating that it is biocompatible. Sehlke et al reported that, upon histologic evaluation, the OsteoCrete appeared to be inert and did not result in an increased inflammatory response.[13] OsteoCrete does not contain any of the hormones or proteins that are needed for hematopoietic cells to recognize and initiate osteoclast differentiation and active resorption, such as PTH (parathyroid hormone), RANKL (Receptor Activator of Nuclear factor-Kappa Ligand), osteopontin (OPN), CSF-1 (Colony Stimulating Factor-1), or MCP-1 (monocyte chemoattractant protein-1).[63,64] RANKL is produced by osteoblasts naturally and in response to PTH. Osteoclast precursors express the RANK receptor that binds RANKL and causes the fusion and differentiation of osteoclasts. OsteoCrete also lacks the proteins that are necessary to stimulate bone formation by osteoblasts, such as osteopontin (OPN), sclerostin, and collagen type I.[64] Without the necessary proteins and hormones to induce osteoclasts and osteoblasts, OsteoCrete mainly serves as a bone filler that is very slowly absorbed and replaced by bone.

In the layer of bone around the experimental and control MSIs, there was no difference in bone volume fraction or bone mineral density. The experimental group had a slightly higher bone volume fraction and bone mineral density than the control group, but the difference was not statistically significant. This can be explained by the fact that OsteoCrete is denser than cortical and especially trabecular bone, due to the high mineral

content, and it solidifies as one large piece of cement with few spaces compared to trabecular bone. In the present study, bone volume fraction and bone mineral density were not good measures of MSI stability due to the inability to differentiate between the OsteoCrete and bone.

Clinical Implications

The clinical implications of this study are that OsteoCrete does not make MSIs more stable. It makes them less stable. OsteoCrete could be used as a bone filler in a sterile orthopedic environment, where it can slowly be absorbed and replaced with bone. However, it is replaced much too slowly to be beneficial in MSI and dental implant applications. MSIs and dental implants are generally loaded much sooner than the replacement of OsteoCrete allows, resulting in failures due to the lack of adequate osseointegration. If proteins that induce osteoclast and osteoblast formation could be added to OsteoCrete, it may serve as a better adjunctive to be used with MSI and dental implant placement.

Limitations

There were some limitations of the present study that were not foreseen. Initially, it was difficult getting the OsteoCrete to predictably flow through the needle used for injection into the MSI pilot hole. The OsteoCrete granules had to be ground finer and more saline had to be added. The smallest needle that would allow the new formulation of OsteoCrete to flow through was a thin-walled 18 gauge BD needle. The

outer diameter of this needle was approximately 1.1 mm, which was the sames size as the inner diameter of the MSI. This resulted in having to push the needle through the hole in the mucosa, the hole in the cortical bone, and into the medullary space. All of the MSIs were placed in slightly movable, non-keratinized mucosa, which also made it difficult to position the needle and find the hole in the cortical bone. For these reasons, there were some experimental sites that did not receive very much OsteoCrete if any. Another limitation of this study was the generalized gingival inflammation around the MSIs that occurred. This resulted in having to remove the gingival tissue in order to screw the SmartPeg into place for Osstell ISQ measurements. Initially, a 12 blade scalpel was used first to remove the gingival tissue. Due to difficulties encountered, an electrosurgery unit was used in most instances for gingival tissue removal.

CHAPTER III

CONCLUSIONS

From the present study, the following conclusions can be made:

1. The MSIs were most susceptible to failure between weeks 1 and 4.
2. Secondary stability is lower with the use of OsteoCrete and does not approach initial stability values after 9 weeks.
3. There was not any bone formation within the OsteoCrete, however there was bone formation around the OsteoCrete.
4. OsteoCrete is biocompatible, but does not actively remodel.
5. μCT bone volume fraction and bone mineral density are not good measures of MSI stability due to the inability to differentiate between OsteoCrete and bone.

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APPENDIX A

Table 1. Osstell Implant Stability Quotient (ISQ) Weekly Measurements. Dog number, E/C: Experimental/Control, Side: Lt=Left or Rt=Right, Site: C=Canine or PM=Pre-molar, X=MSIs that failed.

Dog	E/C	Side	Site	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9
A	E	Lt	PM2	39	28	25	25	25	28	28	28	28	28
A	C	Rt	PM2	32	32	30	32	32	35	35	35	30	22
A	E	Lt	PM2/PM3	30	22	22	20	22	20	20	20	20	20
A	C	Rt	PM2/PM3	28	25	25	28	25	28	30	30	30	28
A	E	Lt	PM3	22	20	20	20	20	17	17	20	20	20
A	C	Rt	PM3	22	22	20	22	22	22	22	22	22	22
A	E	Lt	PM4	25	17	22	25	25	22	25	22	25	25
A	C	Rt	PM4	25	25	25	28	28	30	30	30	28	30
B	E	Rt	PM2	44	42	35	39	25	22	20	20	22	22
B	C	Lt	PM2	35	32	28	28	28	28	25	28	25	28
B	E	Rt	PM3	10	28	X	X	X	X	X	X	X	X
B	C	Lt	PM3	22	22	20	17	20	20	20	20	20	20
B	E	Rt	PM4	25	25	20	20	20	22	22	22	22	25
B	C	Lt	PM4	25	22	22	22	20	20	20	20	22	22
C	E	Lt	PM2	30	30	25	25	22	22	22	25	25	25
C	C	Rt	PM2	30	22	22	25	25	28	28	28	28	30
C	E	Lt	PM2/PM3	22	22	22	22	22	22	22	22	22	22
C	C	Rt	PM2/PM3	28	22	25	25	28	28	22	28	28	28
C	E	Lt	PM3	7	X	X	X	X	X	X	X	X	X
C	C	Rt	PM3/4	10	10	14	17	20	20	20	17	22	35
C	E	Lt	PM4	20	15.5	X	X	X	X	X	X	X	X
C	C	Rt	PM4	22	22	25	22	25	25	22	22	25	25
D	E	Rt	C/PM1	35	30	22	22	22	22	22	25	22	22
D	C	Lt	C/PM1	28	17	7	X	X	X	X	X	X	X
D	E	Rt	PM2	20	25	22	22	22	22	22	22	22	20
D	C	Lt	PM2	28	25	22	22	22	25	25	25	25	25
D	E	Rt	PM3	14	10	7	7	7	7	3	0	3	3
D	C	Lt	PM3	14	10	X	X	X	X	X	X	X	X
D	E	Rt	PM4	20	25	20	22	22	20	20	20	20	20
D	C	Lt	PM4	22	20	17	17	17	17	17	17	17	17
E	E	Lt	C/PM1	25	10	X	X	X	X	X	X	X	X
E	C	Rt	C/PM1	28	20	20	17	22	20	22	22	22	25
E	E	Lt	PM2	22	20	20	20	22	22	22	22	22	22
E	C	Rt	PM2	22	20	22	22	25	25	25	25	25	25
E	E	Lt	PM3	20	14	10	10	10	10	10	10	10	10
E	C	Rt	PM3	17	14	7	7	X	X	X	X	X	X
E	E	Lt	PM4	17	7	14	14	17	17	17	17	20	20

Table 1 Continued.

Dog	E/C	Side	Site	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9
E	C	Rt	PM4	20	14	20	17	17	17	17	17	20	20
F	E	Rt	C/PM1	32	25	14	X	X	X	X	X	X	X
F	C	Lt	C/PM1	30	20	14	14	17	20	20	20	18.5	17
F	E	Rt	PM2	25	25	25	25	25	28	28	28	28	28
F	C	Lt	PM2	30	28	28	30	28	28	30	28	30	30
F	E	Rt	PM3	14	14	7	7	10	14	20	20	20	20
F	C	Lt	PM3	14	10	10	14	14	14	14	14	17	14
F	E	Rt	PM4	20	17	20	22	22	22	22	25	25	22
F	C	Lt	PM4	17	14	17	20	20	20	21	22	22	22

Table 2. Chi-Square for Control and Experimental MSIs Success and Failures.

	Success	Failure	
MSIs	18	5	Chi-square statistic = 0.6053
OsteoCrete + MSIs	20	3	P-value = 0.437

Table 3. Osstell IDx Implant Stability Quotient (ISQ) Statistics. Average weekly ISQ values for the Control and OsteoCrete groups and paired samples T-test.

	Control		OsteoCrete		Side Differences	
Week	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**P-value**
0	23.87	6.41	23.39	8.80	0.48	0.636
1	20.35	6.27	21.43	8.10	-0.61	0.632
2	20.00	6.44	19.57	6.78	-0.22	0.852
3	21.24	6.16	20.39	7.48	1.31	0.299
4	22.75	4.72	20.00	5.48	2.00	0.023
5	23.50	5.30	19.94	5.35	3.00	0.010
6	23.25	5.25	20.11	5.88	2.27	0.059
7	23.50	5.45	20.44	6.55	2.40	0.070
8	23.83	4.23	20.89	5.95	1.87	0.055
9	24.25	5.26	20.78	5.95	1.60	0.187

APPENDIX B

Figure 1. MSI and Osstell IDx Components. (a) Neodent 7mm SLA MSI (b) MSI Hand Driver (c) Osstell IDx (d) Transducer (e) SmartPeg Type A3 (f) SmartPeg Wrench

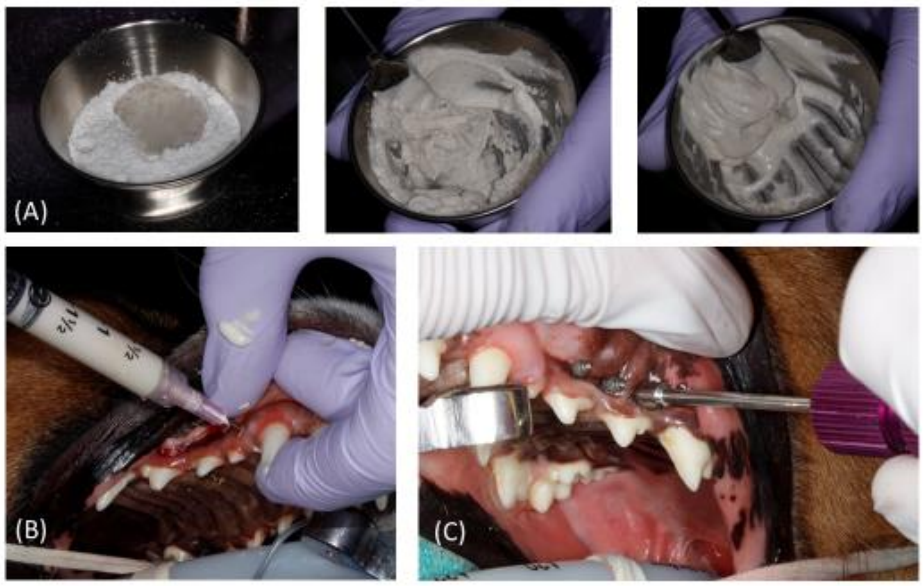

Figure 2. OsteoCrete Mixing and Placement with MSI. (A) OsteoCrete Mixing. (B) OsteoCrete Injection (C) MSI Placement Using Hand Driver

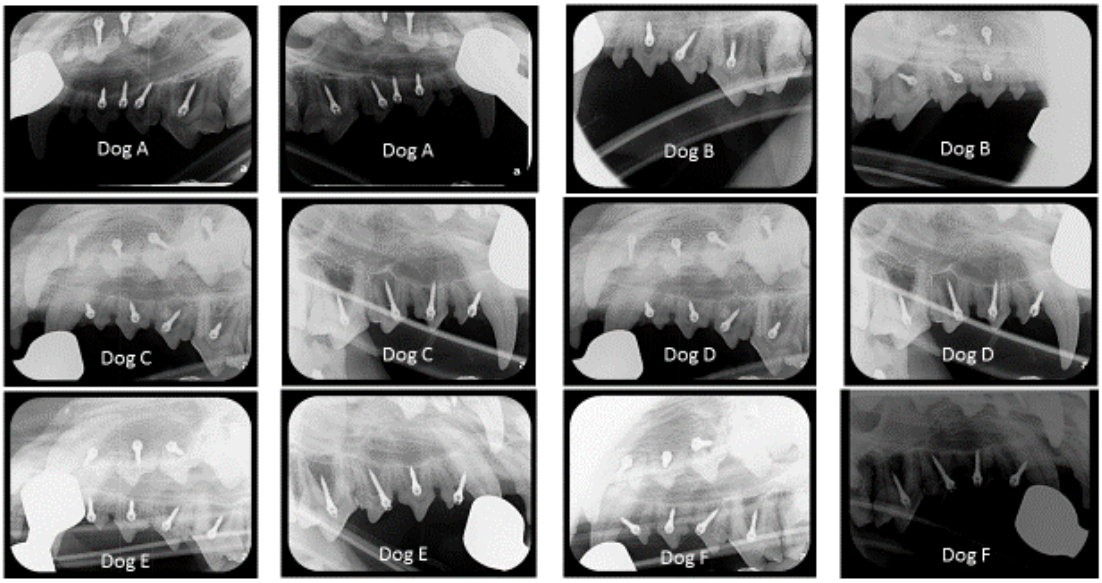

Figure 3. Post-op MSI Placement Radiographs.

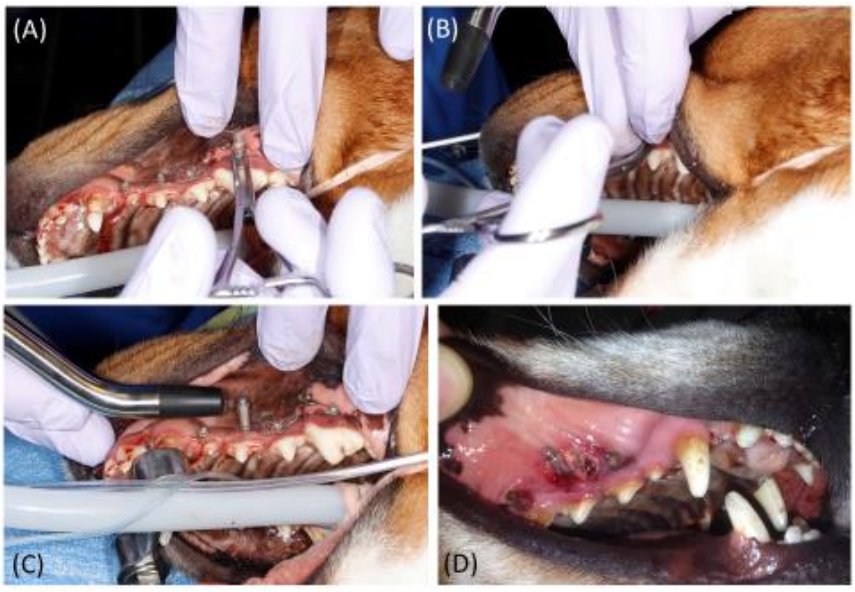

Figure 4. ISQ Measurements. (A and B) SmartPeg Type A3 Placement. (C) Osstell ISQ Measurements. (D) Gingival Inflammation.

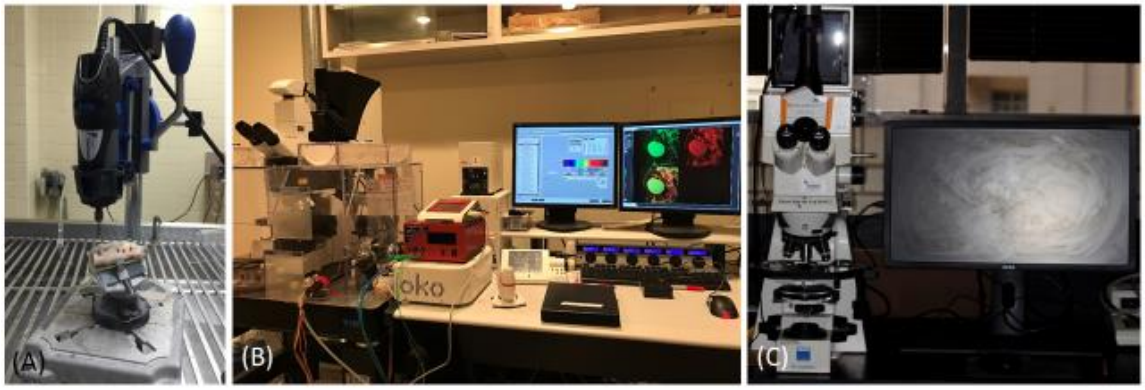

Figure 5. Trephine and Microscopes. (A) Trephoning of MSI Samples. (B) Confocal Microscope. (C) Light Microscope.

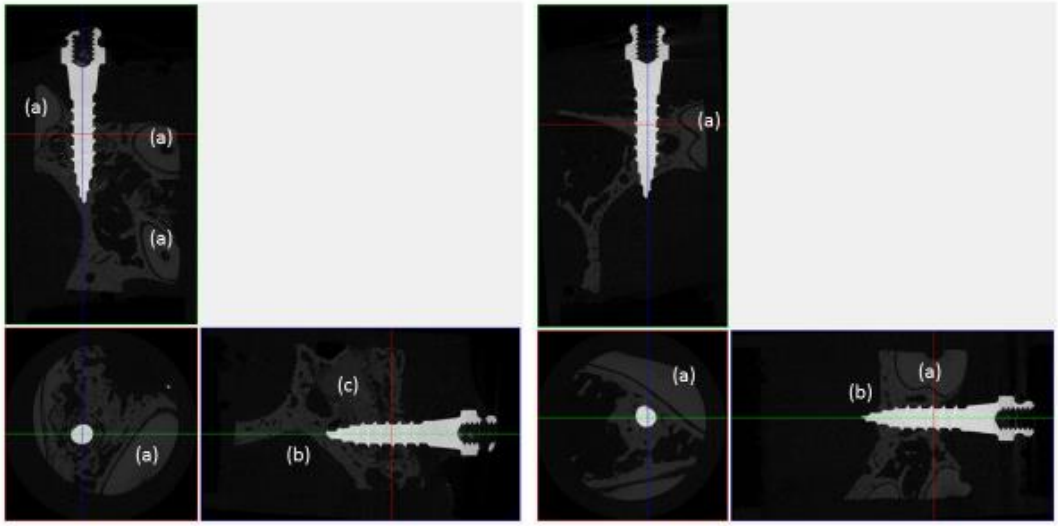

Figure 6. µCT Analysis. X,Y,Z orientations showing MSI within maxillary bone between tooth roots (a). The apical portion of most MSIs were within the maxillary sinus (b). The buccal cortical bone is the side towards the head of the MSIs. The left three orientations are from the experimental group and OsteoCrete (c) can be seen in the lower right orientation. The right three orientations are from the control group.

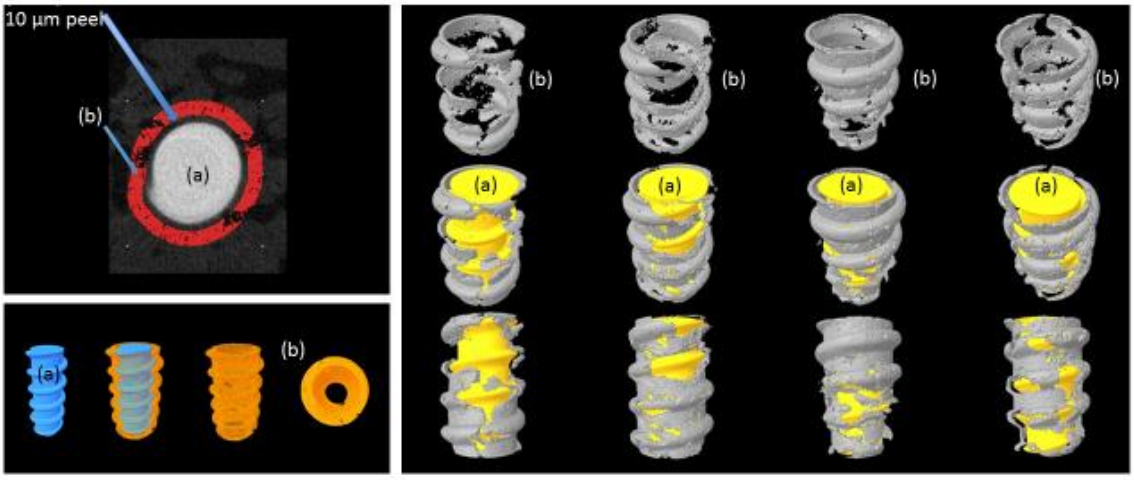

Figure 7. µCT 3D Renderings and ROI. MSI (a) was segmented out and 3D ROI/VOI (b) was 10-20 µm around each MSI. The two middle ROI's are experimental and the outer two are controls, which little difference can be observed.

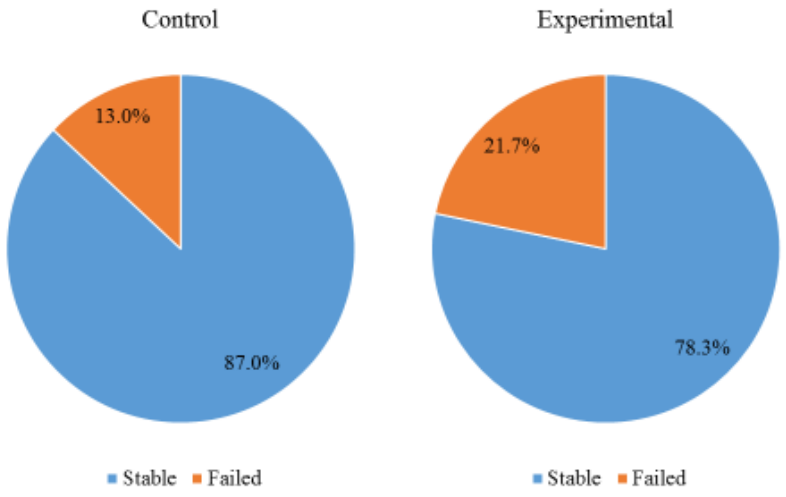

Figure 8. Control and Experimental Success and Failure Rates.

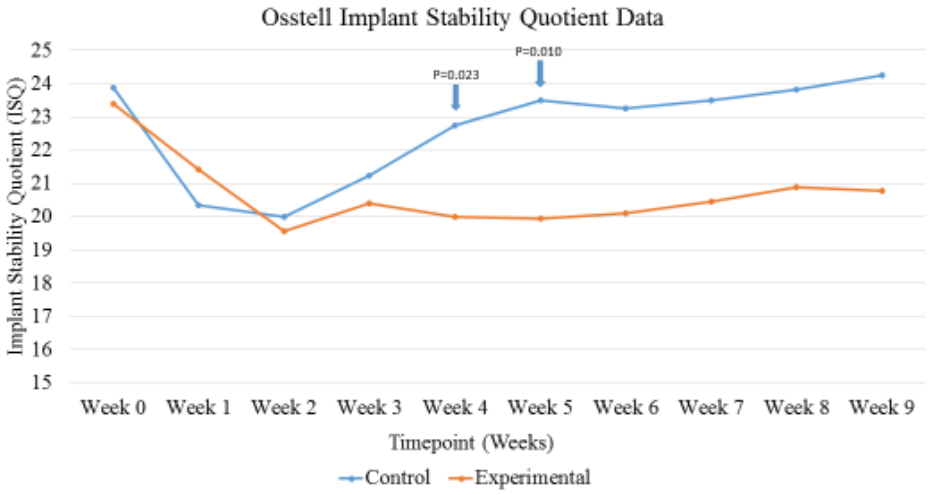

Figure 9. Osstell Implant Stability Quotient Data. Control vs. Experimental.

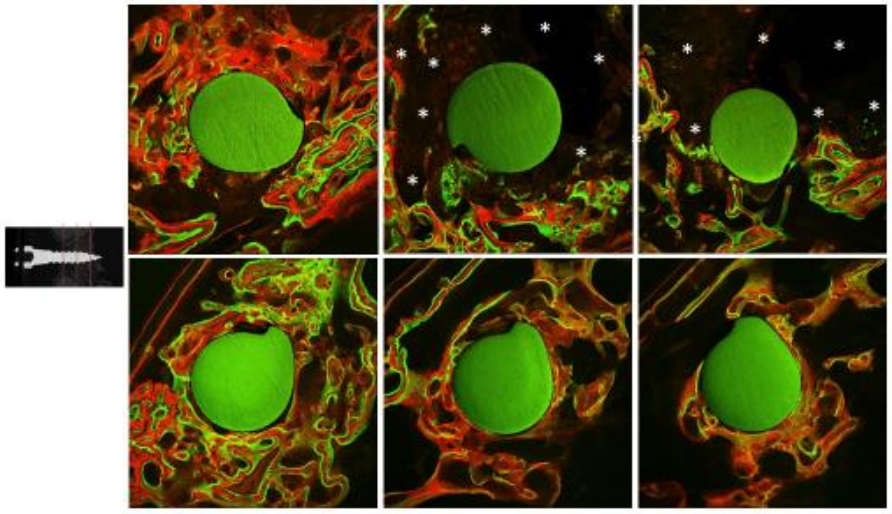

Figure 10. Confocal Fluorescent Histological Images. The upper row of images is from the experimental group and shows OsteoCrete (*) spread and lack of bone mineralization within the OsteoCrete around the MSI. The lower row of images is from the control group and shows bone-to-implant contact and normal bone mineralization around the MSI. Within each row, the left two images are cortical sections, middle two images are mid trabecular sections, and the right two images are apical trabecular sections as depicted by the MSI image on the left.

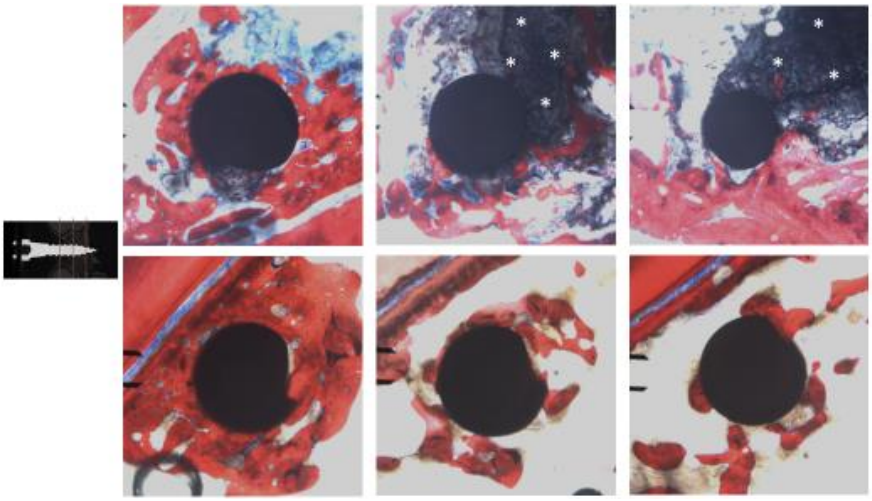

Figure 11. Stevenel's Blue Histological Images. The upper row of images is from the experimental group and shows OsteoCrete (*) spread and lack of bone mineralization within the OsteoCrete around the MSI. The lower row of images is from the control group and shows bone-to-implant contact and normal bone mineralization around the MSI. Within each row, the left two images are cortical sections, middle two images are mid trabecular sections, and the right two images are apical trabecular sections as depicted by the MSI image.

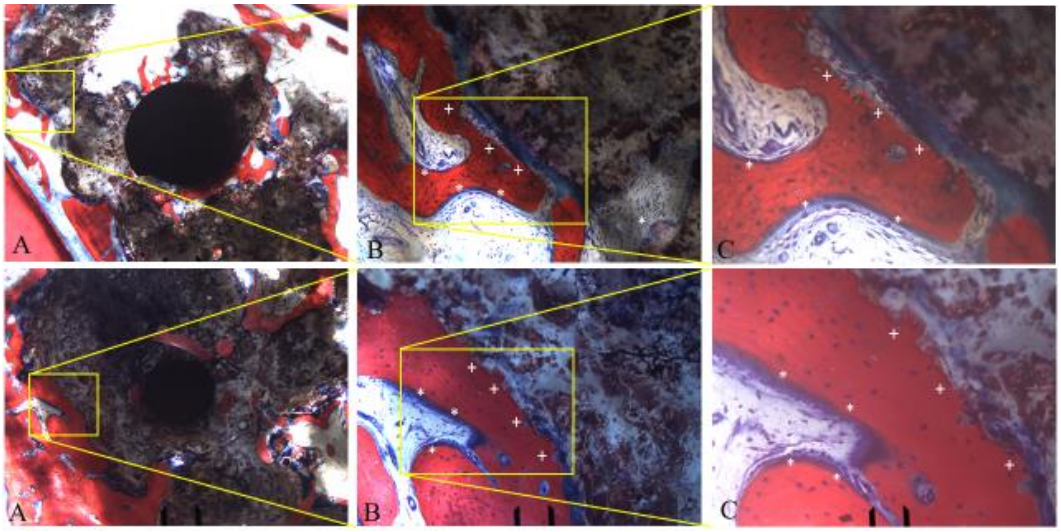

Figure 12. Stevenel's Blue Experimental Slide. (A) 2.5X Magnification. (B) 10X Magnification. (C) 20X Magnification. The (*) points out the osteoblast layer and the (+) points out the jagged bone along the side with OsteoCrete where bone was laid down up to the OsteoCrete.

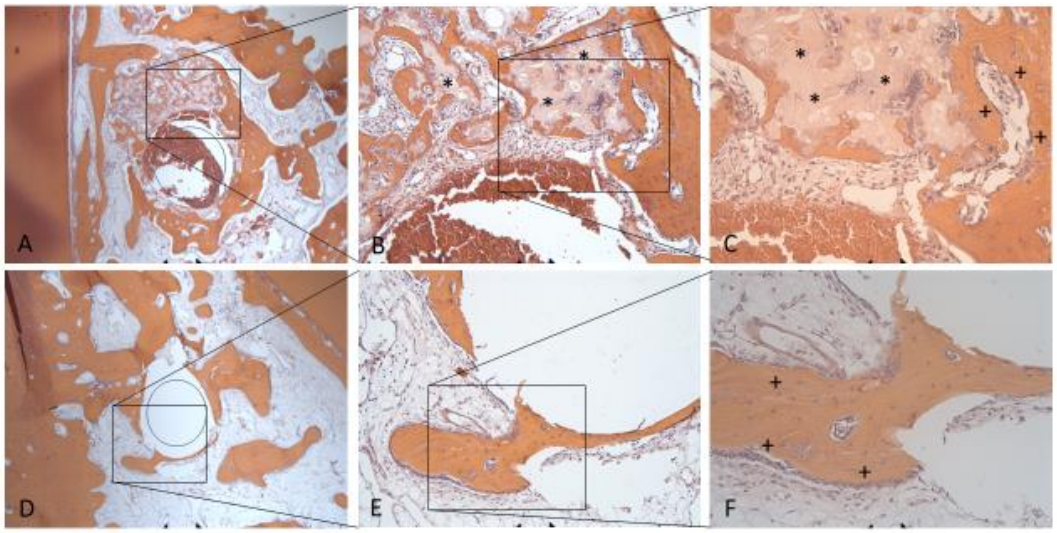

Figure 13. H&E Slides. Experimental (A) 2.5X Magnification. (B) 10X Magnification. (C) 20X Magnification. Control (D) 2.5X Magnification. (E) 10X Magnification. (F) 20X Magnification. The (*) points out where OsteoCrete was present and the (+) points out where osteoblasts were present.

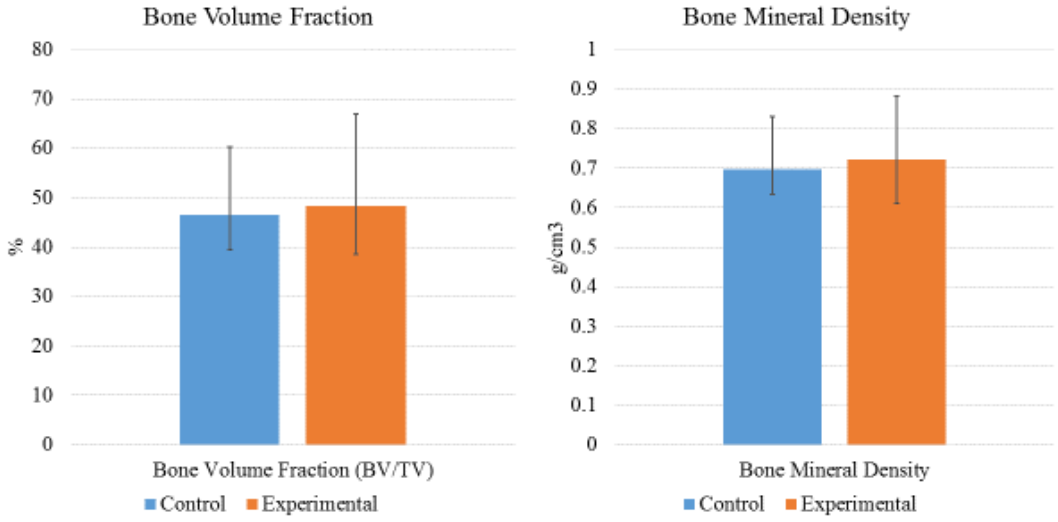

Figure 14. µCT Bone Volume Fraction and Bone Mineral Density. Control vs. Experimental.