INFLUENCE OF SLA SURFACE FINISH ON MSI STABILITY WHEN PLACED ALONG WITH OSTEOCRETE BONE CEMENT

A Thesis

by

AUSTIN SIDNEY HODGES

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Phillip M. Campbell
Committee Members,	Peter H. Buschang
	Larry P. Tadlock
	Elias D. Kontogiorgos
Head of Department,	Larry L. Bellinger

May 2017

Major Subject: Oral Biology

ABSTRACT

The purpose of this study will be to compare the stability of 7 mm sandblasted, acid-etched (SLA) titanium miniscrew implants (MSIs), to 7 mm machine polished titanium MSIs.

Using a randomized, split mouth design in six skeletally mature male beagle dogs, 28 machine polished MSIs placed with OsteoCrete were compared to 28 SLA MSIs placed with OsteoCrete. Both groups of MSIs were placed along with a magnesium bone cement (OsteoCrete). Osstell ISQ measurements of MSI stability were taken weekly for nine weeks. Bone volume fractions and bone mineral densities of the layer of bone 10-20 μm from the MSIs were evaluated using micro-computed tomography (μCT). Histology was used to evaluate new bone formation and the bone cement-to-MSI interface.

The control and experimental MSIs had a success rate of 93.1% and 100%, respectively. The groups had nearly identical ISQ values at the time of placement. The decrease in ISQ values during the first 2 weeks was significantly ($p=0.024$) greater in the experimental than control MSIs. Difference in ISQ values continued to increase between week 2 and 8, with statistically significant ($P<0.05$) differences at weeks 5, 6, 7, and 8. The differences in bone volume fraction (BV/TV) and bone mineral density (gHA/cm^3) were not statistically significant between the two groups. Immunofluorescence showed no new bone within the OsteoCrete, nor along the MSI surface when OsteoCrete was present. There was new bone around and up to the edge of

the OsteoCrete. Osteoblasts were evident in trabecular bone, but not adjacent to regions filled with OsteoCrete. The H&E sections showed areas of acellular bone extending approximately 0.25 - 0.5 mm from the MSI. There were minimal Howship's lacunae and osteoclasts noted, as well as minimal inflammatory cells present.

SLA MSIs placed along with OsteoCrete had decreased primary and secondary stability. OsteoCrete is biocompatible but it is slowly resorbed and inhibits the normal healing process that is expected to occur around MSIs.

DEDICATION

This work is dedicated to my parents, David and Jan Hodges, and brothers, Patrick and Preston Hodges, for their constant love, encouragement, and support. Without the work ethic and drive my parents instilled in me, I would not have made it where I am now.

ACKNOWLEDGEMENTS

I would like to thank my committee, Dr. Peter Buschang, Dr. Campbell, Dr. Tadlock, and Dr. Kontogiorgos, for their advice and guidance on research, orthodontics, implants, and bone cement background. I would also like to thank Gerald Hill for his help with the surgeries and excellent dog care. I am also thankful for Connie Tillberg's help with histological slide preparation and Dr. Lynne Opperman's help interpreting and reading slides. I would also like to thank my co-resident and twin brother, Patrick Hodges, for his support learning the intricacies and skills involved with animal projects from the surgical procedure, sample harvesting, and data analysis. Lastly, I appreciate the Class of 2017 (Patrick Hodges, Charlene Sugay, Kate Preston, Blerta Abdi, and Mike Ross) for making the last three years enjoyable during our orthodontic training.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a thesis committee consisting of Dr. Peter Buschang, Dr. Phillip Campbell, Dr. Larry Tadlock in the Department of Orthodontics and Dr. Elias Kontogiorgos in the Department of Restorative Sciences. All other work conducted for the thesis was completed by Austin Hodges, independently.

Funding Sources

This graduate study was supported by the Robert E. Gaylord Endowed Chair from the Baylor Oral Health Foundation.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

One of the most important considerations in orthodontics is anchorage. While there are many forms of anchorage that orthodontists have at their disposal, miniscrew implants (MSIs) have become an ideal anchorage option as they offer near absolute anchorage and require minimal patient compliance. The main problem with MSIs today is their relatively high failure rates.[1,2] The loss of bone-to-implant contact, which changes during the primary and secondary stability phases has been reported to be the most common cause of MSI failure.[3] Other causes of MSI failure include trauma to the MSI causing bone fracture and pull out. A systematic review reported a failure rate of 16.4% for MSIs.[2] In a retrospective study the MSI failure rate was found to be 11.4%.[1] Failure rates of MSIs are higher than rates of endosseous implants which has been reported in a systematic review to be 5.4% at 10 years.[4] The relatively high failure rates of MSIs contribute to orthodontists avoiding their use. MSI failure often delays treatment, as it requires replacing them in different locations, which are sometimes not available, or placing them in the same location after healing occurs. One of the ways that failure rates could be reduced is by using sandblasted and acid-etched (SLA) MSIs.

SLA treatment increases the area of the implant surface, which results in increased secondary stability because it provides greater bone-to-implant contact.[5] SLA treated endosseous implants have become the gold standard in dentistry due to the

improved healing characteristics associated with them.[6-8] Multiple studies have shown that SLA MSIs provide increased removal torque and bone-to-implant contact due to increased osseointegration resulting in higher success rates and increased stability.[5,9,10] SLA MSI stability could be further enhanced by increasing their primary stability.

Primary stability could be enhanced if: 1) a material that hardens could be introduced into the trabeculation space and 2) that material adheres to the MSI immediately after insertion. While polymethylmethacrylate (PMMA) accomplishes both of these goals, it is bioinert and has no potential for bone remodeling or osseointegration, which is necessary for the stability of MSIs. OsteoCrete is a newly introduced magnesium based bone cement that reportedly can be used as a bone cement or bone filler because it has osteoconductive properties.[11-14] OsteoCrete has been shown to increase the stability of bone screws through its bone-to-stainless steel adherence properties.[12] To date, the potential use of OsteoCrete with intraorally placed MSIs remains unexplored. Hirvinen et al performed a study evaluating the influence of OsteoCrete on bone-screw interfaces in the third metacarpals and third metatarsals of horses.[12] The use of a magnesium-based cement increased the peak torque to failure when compared to both the calcium-based cement and the control group. However, the magnesium-based cement was not resorbed after 7 weeks.

The present study will be the first to evaluate whether increasing the surface area of MSIs placed in OsteoCrete bone cement increases their stability and improves their survival rates. This study will longitudinally compare the stability of SLA finished MSIs to machine finished MSIs, with both groups of MSIs placed into OsteoCrete. If

the working hypothesis is correct (i.e. that the increased surface area and mechanical retention of the SLA finished MSIs placed along with OsteoCrete provides increased primary and secondary stability), it should improve the survival rate of MSIs. This would provide orthodontists a more reliable way to place MSIs and give them more confidence when planning cases that require maximum anchorage.

LITERATURE REVIEW

History and Significance of MSIs

The idea of absolute anchorage in order to obtain complete stability of the reactive unit with the use and application of miniscrew implants was first described in 1945 by Gainsforth and Higley.[15] Gainsforth and Higley used a 2.4 mm pilot hole for 3.4 mm x 13 mm vitallium screws that they placed in the ascending ramus of 6 dogs. They used the vitallium screws for anchorage to retract maxillary canines with orthodontic elastics that delivered between 140 and 200 g of force. The system allowed for tooth movement to occur but, unfortunately, all of the screws had failed by day 31. The authors suspected the implant failure to be related communication with the oral environment and the screws exposure to pathogens. It was also possible that the dog's bodies were rejecting the vitallium metal via a localized immune reaction, which may have resulted in changes to the supporting bone.

The biocompatibility properties of Titanium provides a solution to the vitallium rejection. In 1952, Per-Ingvar Branemark conducted a vital microscopic study of the bone marrow of the rabbit fibula and discovered that the titanium oculars used could not

be removed from the bone after the healing period.[16] This suggested that titanium is biocompatible and able to integrate with bone termed osseointegration. During the 1960s, Branemark performed subsequent studies using endosseous titanium implants that further demonstrated biocompatibility and osseointegration.[17] These studies found that bone grew into the tiny spaces and was very closely adapted to the titanium. After this discovery, the use of endosseous titanium implants in prosthodontics began to gain traction and the use of titanium implants in orthodontics slowly emerged.

The use of endosseous implants in orthodontics was initially reported by Roberts et al in 1989.[18] Following this publication several more studies using endosseous implants as orthodontic anchorage demonstrated their utility.[18-26] While these studies proved that endosseous implants could be used for skeletal anchorage, there were limitations that inhibited their widespread adoption in orthodontics. These limitations included non-ideal placement sites in the retromolar or edentulous area associated with less ideal force vectors, invasive surgical placement and removal, and delayed loading period.[27,28]

The first clinical report of the use of a miniscrew in a human patient in orthodontics was published in 1983 by Creekmore and Eklund.[29] A 13 mm surgical vitallium bone screw was placed beneath the anterior nasal spine on a mature female and successfully utilized to intrude her upper incisors 6 mm and correct her severe overbite. The screw remained stable throughout treatment. While the successful use of a vitallium miniscrew was reported the use of miniscrews would not catch on until some years later.

In 1997, Kanomi et al published a protocol using smaller screws.[27] They were 1.2 mm x 6 mm titanium screws designed for the fixation of bone plates in craniofacial reconstructive surgery. These screws were small enough to be placed interdentally between the mandibular central incisors and used to intrude the mandibular incisors over a four month period. Their smaller size and the less invasive surgical placement led to the beginning of the miniscrew acceptance in orthodontics. However, it wasn't until Costa et al developed a more simplified placement protocol in 1998 that MSIs actually became widely accepted.[28] In 2001, Ohmae et al completed a study attempting to intrude the mandibular posterior teeth in beagle dogs.[30] After achieving an average 4.5 mm of intrusion, they evaluated the ease of MSI removal using a manual screw driver. Since the introduction of the smaller and more simplified MSI protocol numerous studies have demonstrated the benefits of using MSIs for skeletal anchorage leading to more predictable anchorage control.[27,28,31-35]

MSI Failure

While MSIs have gained widespread acceptance and popularity through the orthodontic community, MSI failure rates are higher than that of its larger relative, the endosseous implant. In order for MSIs to be successfully used they must remain stable the entire period of time that skeletal anchorage is desired.

A systematic review of endosseous implants completed by Moraschini et al found a 10 year mean survival rate of 94.6% (i.e. 5.4% failure rate).[4] A total of 23 articles were included in the review, evaluating a total of 7711 implants: ten prospective, nine retrospective, and four randomized clinical trials all of which had a follow up of at

least 10 years. KC Nixon et al performed a retrospective study evaluating 5 and 10 year success rate of 1000 SLA endosseous implants placed in private practice.[36] They found the failure rate to be 6.9% at 5 years and 9.1% at 10 years. Daniel Buser et al retrospectively analyzed 511 SLA titanium endosseous implants and found a 10 year failure rate of 3.0%.[37]

The success rates for MSIs do not yet compare to the relatively high success rates of endosseous implants.[38-40] A systematic review completed by Crismani et al found a mean MSI success rate of 83.8% (i.e. failure rate of 16.2%), with a standard deviation of 7.4%.[41] Systematic reviews conducted by Shatzle et al and Reynders et al. also found mean MSI success rates between 83.6% and 80%, respectively (i.e. failure rates of 16.4% and 20%, respectively).[2,42] Shatzle et al reviewed 27 studies which included a total of 2374 MSIs.

There are multiple factors that contribute to MSI failure. Host factors such as osteoporosis, uncontrolled diabetes, and smoking increase the risk for implant failure.[43] Operator factors affecting failure rate include: placement technique, root contact, thermal insult to bone, pilot hole, placement location (keratinized vs. non-keratinized tissue), and excessive loading.[38,40,44-49] In order to understand how this relatively high failure rate can be reduced we must first understand the process of bone healing around the MSIs and what steps can be taken to improve the success MSIs.

A survey performed by Buschang et al in 2008 found that the percentage of MSI failures was significantly related to a number of factors.[50] The orthodontists' experience measured by either the number of miniscrews they had placed or the number of years

they had been using them was statistically significant. The percentage of failures reported was significantly lower for orthodontists who inserted their own MSIs than for those who referred the placement to oral surgeons or periodontists. The respondents who used periapical or cone-beam radio-graphs to determine placement sites reported lower failure rates than those who used panoramic radiographs, lateral cephalograms, or nothing. The orthodontists who had fewer failures were more satisfied with MSIs and also believed that MSIs had made their treatment faster and better. Those who were satisfied or very satisfied with MSIs had been using them significantly longer than those who were not satisfied. This article provides evidence that MSI use in clinical orthodontic treatment has become the norm rather than the exception.

Bone Healing and Osseointegration

The bone healing events that occur around MSIs after placement are nearly identical to those that occur in normal bone wound healing. These events can be broken down into four main categories: hematoma, clot resolution, osteogenic cell migration (osteoconduction), de novo bone formation.[51] The initial two phases after MSI placement are blood clot formation and resolution. The third and most important healing phase, osteogenic cell migration relies on the recruitment of bone forming cells through the blood clot. The platelet activation results in this osteogenic cell migration. The fourth healing phase, de novo bone formation, results in an interface between the implant and bone that is mineralized. De novo bone formation can occur through distance osteogenesis where the osteoblast is polarized to lay down bone matrix on the surface of old bone and contact osteogenesis where the osteoblast is polarized in the opposite

direction to lay down bone matrix on the implant surface. Contact osteogenesis is the ideal de novo bone formation for bone to implant contact and relies on implant topography and osteogenic recruitment for this to occur. The final healing phase which Davies does not address is bone remodeling. It is through de novo bone formation that true bone-to-implant-contact (BIC) occurs. This BIC leads to the long-term stability and success of the MSI.[52]

Berglundh et al studied the bone formation process adjacent to endosseous implants in 20 labrador dogs and a total of 160 endosseous implants.[52] They evaluated healing between 2 hours and 12 weeks using ground sections and decalcified sections. At 4 days there was coagulum with a multitude of fibroblast-like cells surrounding vascular structures which transitioned to a provisional matrix containing newly formed woven bone with osteoblasts and osteoclasts lining the trabeculae at 1 week. At 2 weeks the woven bone extended from the implant surface to the parent bone. At 4 weeks the newly formed bone appeared to contain both parallel-fibered and lamellar bone. Bone was projecting along the SLA surface. At 6 weeks most of the experimental chambers were filled with bone and generally had parallel-fibered and lamellar bone. From 6 to 12 weeks marked signs of remodeling were visualized within the experimental chambers.

When a miniscrew is placed there is a traumatic insult and the bone healing sequence begins. The miniscrew goes through a phase of primary stability which decreases through the third to fourth week and rises into secondary stability once bone healing and osseointegration occurs.[3]

Primary Stability

Primary stability is achieved primarily through mechanical retention between the MSI and bone contact at the time of placement. This stability is primarily related to the cortical bone.[53] There are four factors that play a role in the primary stability: bone quality, implant design, placement protocol, and immediate loading.[54]

Bone Quality

Cortical bone thickness is the most important contributor to bone quality as it relates to MSI stability. An increase in cortical bone thickness has been associated with increased BIC, pullout strength, and insertion torque (i.e. increased primary stability).[54-62] Although increased insertion torque increases primary stability, too much insertion torque can result in screw breakage during placement as well as micro fractures and bone damage.[47,54,63-65] This bone damage can have a detrimental effect on the stability curve as the bone breaks down and remodels, resulting in micro motion of the MSI and an inability to attain secondary stability.[66]

Bone density also plays an important role in regards to primary stability. It has been shown that bone density is related to pullout strength and insertion torque.[54,56,61] This is also related to higher initial BIC. Hung et al placed MSIs in synthetic bone fabricated with cortical densities of 0.8 g/cc and 0.64 g/cc and measured the differences in insertion torque and pull out strength between the two groups.[67] The denser, 0.8 g/cc, group resulted in significantly higher insertion torque (156% increase) and pullout strength (135% increase). While the pullout strength was increased, the insertion torque

had a higher increase which may not be desirable if it increases so much that it results in micro fractures to the surrounding bone.

Implant Design

In 1968, Ansell and Scales evaluated the effect of screw length, shape, diameter, and thread design on implant failure.[68] They found that less insertion torque was key to decreasing implant failure and recommended using pilot holes with a torque limiting driver. Today's improved titanium screws have resulted in less breakage and therefore limiting insertion torque may be less of a concern.

Wilmes et al found that MSIs with a larger diameter and a conical shape produced in higher insertion torque than MSIs with smaller diameters and rectangular shape.[54] Lim and Hong also found that longer MSIs with larger diameters produced higher insertion torques.[69]

Brinley et al studied the effects of thread design and fluting on insertion torque and pullout strength using a cadaver and synthetic bone model.[70] The synthetic bone model showed that decreased thread pitch increased pullout strength. Both cadaver and synthetic bone models indicated that fluted MSIs had significantly higher insertion torque and pullout strength.

While SLA surface characteristics do not have a direct effect on primary stability, placing MSIs with a roughened surface in bone cement such as OsteoCrete should increase their primary stability via mechanical retention of the OsteoCrete within the roughened surface.

Placement Protocol

Bone can become overheated when placing pilot holes, leading to necrosis. Eriksson and Albrektsson established that temperatures above 47 degrees Celsius can cause osseous necrosis.[71] It is believed that irrigating while drilling the pilot hole helps control the temperature and reduces the likelihood of overheating the surrounding bone.

MSI placement should be performed with as little trauma as possible in order to decrease the amount of boney remodeling and healing required during the crucial primary stability phase. Traumatized bone must be remodeled. It is laid down as woven bone initially which is less dense and does not provide as much stability as undamaged mature lamellar bone does.

Carrillo and Buschang developed a guide to implant placement technique which reduced their failure rate to 4% for both maxillary and mandibular MSIs.[72,73] Bone condition related to adequate interradicular space, cortical bone thickness of at least 1 mm, alveolar crest height which may require inserting the MSI at an apically directed angle. Tissue type related to placing MSI in attached gingiva or the mucogingival junction with a cleansable attachment in order to decrease inflammation. Radiographic evaluation of implant site allows one to assess implant site prior to placement. Anatomic structures to avoid include: greater palatine foramen and neurovascular bundle, incisive canal and foramen, midpalatal suture in growing patients, nasal floor, maxillary sinus, mental foramen, and the mandibular canal. The steps for MSI placement Carrillo and Buschang recommend are: 30 second chlorhexidine rinse, locate the insertion site,

anesthetize the patient, measure the tissue depth, place the MSI tip into the insertion site, assess the insertion path, insert the MSI, check for primary stability.[73]

Immediate Loading

Endosseous dental implants with rough surfaces are more likely to be successful when used in immediate loading situations.[74-76] Although this concept of immediately loading may also apply to MSIs; the majority of studies evaluating the effect of immediately loading MSIs on stability have not found significant difference between unloaded MSIs.[5,9,40,77,78]

Secondary Stability

Secondary stability is largely dependent on primary stability. Increased primary stability results in an increased secondary stability.[79,80] Micromotions above 50-100 micrometers have been shown to have a negative influence on osseointegration and bone remodeling.[80] The micromotions caused bone resorption at the implant interface and the formation of fibrous tissue. This is why primary (mechanical) stability is essential for a successful secondary stability (osseointegration of the implant).

Host Related Factors

Secondary stability is dependent on the bone's ability to heal around the implant interface. The body must recruit osteoblasts resulting in boney deposition around the implant surface for secondary stability to occur. Host related factors that have all been implicated in poor implant stability resulting in failure include: poor oral hygiene, uncontrolled diabetes, smoking, osteoporosis, and parafunctional habits.[38,43,47,81] Bone

density, cortical thickness, and gingival inflammation are host related factors that can also have an impact on MSI failure.[47,82,83]

Placement Protocol

As reviewed in the primary stability section, placement protocol is also crucial for attaining secondary stability. In order to achieve successful MSI placement, one must place the MSI in a way that maximizes primary stability within cortical bone with the least amount of boney damage possible. If primary stability is not obtained, secondary stability is likely to be negatively affected and may not be achieved at all.

Surface Characteristics

SLA treatment increases the surface area of MSIs which results in increased secondary stability because it provides increased bone to implant contact.[5] While many authors agree that sand blasting and acid etching increases the surface area there is no quantification of exactly how much the surface area is increased. Dr. Jason Cope gave a lecture at the 2015 Angle meeting where he stated that surface roughness increases the surface area 200% - 600%.

Buser et al placed hollow cylinder implants in the tibia and femur of miniature pigs to evaluate the influence of the implant surface on bone integration using histomorphometric evaluation.[84] The percentages of bone to implant contact (BIC) were: Electropolished and sandblasted/acid pickled (medium grit; HF/HNO3) surfaces had 20 – 25% BIC, Sandblasted implants with a large grit and titanium plasmasprayed implants had 30 – 40% BIC, sandblasted/acid attacked surfaces (large grit; HCl/H2SO4) had 50 – 60% BIC, and hydroxylapatite coated implants had 60–70% BIC. Although the

hydroxylapatite coated implants had the highest BIC they consistently revealed signs of surrounding boney resorption. They concluded that the extent of bone-to-implant contact is positively correlated with an increased implant surface roughness.

A 12 week study evaluating the rate and degree of osseointegration between SLA and machine polished endosseous dental implants found that the two groups showed similar healing characteristics but that the rate and degree of osseointegration was superior in the SLA group.[7] The results showed that after 1 week of healing, the bone-to-implant contact (BIC%) for the SLA implants was almost twice as high as the machined implants: 24.8% vs. 13.9%. The SLA group reached their peak BIC% of 65% at 4 weeks and remained at this high level through week 12. The machined group BIC% gradually increased throughout the healing period to 36.8% at 12 weeks. This led them to conclude that the SLA surface is a truly "osteophilic" surface resulting in early osseointegration with nearly two times as much bone-to-implant contact than a machine polished surface allows.

A rabbit study comparing 3 different surface topographies of endosseous dental implants showed that implants roughened using 25 μm particles of titanium and 75 μm particles of aluminum oxide resulted in higher removal torques and bone-to-implant contact than turned (polished) implants.[85]

Orton et al performed a study using 6 dogs with bilateral midshaft femoral osteotomies to evaluate fixation with porous titanium bone plate and screws compared to smooth surfaced bone plate and screws.[86] They found a mean removal torque for the porous titanium-surfaced screws to be 32.3 kg x cm which was significantly greater than

the mean removal torque for standard screws at 4.4 kg x cm. They also found an accelerated primary osteotomy gap healing in the group fixed with porous titanium screws using radiographic and histologic evaluation.

Franchi et al placed seventy-two 8 mm x 3.8 mm titanium implants in the tibia of six sheep to evaluate peri implant ostoegenesis using histomorphometric analysis.[8] The implant with the highest BIC and Vickers hardness number was the SLA-60 followed by SLA-120, followed by machined implants. They recommended using SLA-60 implants because the moderately deep titanium cavities resemble the osteocyte lacunae which could act as a microscopic scaffold for mesenchymal and/or osteoblast-like cell adhesion.

Chaddad et al conducted a study comparing insertion torque and survival rate of SLA MSIs to machined titanium MSIs.[39] They found that there was not a statistically significant difference in the survival rate between the two types of MSIs. However, they did not use the same type of MSIs. The diameters and lengths were not consistent between the two groups, which likely introduced bias and problems with internal validity. This study did not measure the stability of the MSIs nor did it measure the removal torque.

Ikeda et al performed a three-dimensional comparison of peri-bone-implant contact of SLA and machined miniscrew implants.[5] They found that SLA surface treatment has significant effects on the bone surrounding the MSIs. Their results showed increased secondary stability of SLA MSIs, which should be related to higher success rates.

Chang et al evaluated the effect of microrough surface treatments and loading on miniscrews using removal torque and histomorphometric analysis.[9] They found no difference between the loaded and unloaded conditions. The SLA and SL/NaOH loaded MSIs had higher removal torques and BIC than the machined MSIs.

Kim et al placed ninety-six MSIs in male beagle dogs to compare total removal energy between SLA and machined MSIs.[10] They found that SLA MSIs had a significantly higher total removal energy value than the machined MSIs which they suggested this indicates osseointegration of the SLA MSIs after insertion.

While SLA surface treatment has been shown to substantially enhance the secondary stability of both endosseous and miniscrew implants, the question that remains to be answered is whether SLA surface treatment can also be used to enhance primary stability, which would further enhance secondary stability.

Assessing Stability through Quantification

Many methods have been evaluated to assess stability and osseointegration of miniscrew implants. The focus will be on the three main methods that will be used in the present study. These include radiofrequency using an osstell mentor, bone volume analysis using micro-computed tomography, and histomorphometric evaluation of the tissue surrounding the miniscrew implant.

Resonance Frequency Analysis

Resonance frequency analysis (RFA) is a noninvasive method used to determine implant stability prospectively in a living subject. RFA quantifies the stability of an implant based on vibrations between the implant and bone. An implant has three

directions of vibration possible: horizontal, vertical, and rotational. Of these three, when it comes to measuring implant stability we are most interested in the horizontal vibrations. These measurable vibrations are created by an electromagnetic field which excites a magnet attached to the implant resulting in micro vibrations creating sound waves.[87] The RFA device then records the sound waves produced and provides a quantitative measurement that may be used to determine stability comparing the values to sequential measurements over time.[88,89]

The newest device in RFA, the Osstell Mentor, has become the gold standard as it applies to measuring sequential implant stability in vivo. The Osstell Mentor creates an electromagnetic signal from the hand piece which excites the SmartPeg magnet attached to the implant. This excitement produces ranges from 5kHz to 15 kHz.[87] This resonance vibration is measured by another transducer located in the hand piece which then displays the implant stability quotient (ISQ). The ISQ provides a quantitative measure for the implant's stability which ranges form 0-100 where 100 is the most stable.[90] The Osstell Mentor meausrements should be taken perpendicular to the implant with a repeated transducer position for the best accuracy and reliability.[91]

Glauser et al used RFA to prospectively evaluate endosseous implant stability over one year in 23 patients with 81 total Branemark System implants.[92] They found that after two months the failing implants had an average ISQ of 43 and the successful implants maintained an average ISQ of 60. This indicated a statistically significant difference in ISQ values between the failing and successful groups. The Osstell Mentor was first shown to be a reliable for measuring MSI stability by Ure et al.[3] It was used to

prospectively measure the stability in a canine split mouth design between MSIs placed in keratinized and nonkeratinized tissue. Since this study, several other MSI studies have reliably used the Osstell Mentor and ISQ values to quantify miniscrew implant stability in vivo.[5,48,93]

Micro-computed Tomography (µCT)

The initial titanium implant studies used histomorphometric analysis to evaluate bone to implant contact as the gold standard.[16,17] Wigianto et al attempted to develop three dimensional models constructed from digitized photographs of a series of two dimensional histological slides to quantify bone to implant contact ratios.[94] While histologogical evaluations allow one to visualize the tissue surrounding the implant at a cellular level, it only provides a single cross section for evaluation which leaves a large majority of the implant unevaluated.[95,96] The destructive nature of histology preparation prevents any further analysis or studies from being performed on the specimens. This is where the benefit of micro-computed tomography (µCT) lies. It makes it possible to assess the bone around the entire implant, without destruction of the specimen.[96] Muller et al performed a study comparing µCT to that day's gold standard, histology, and determined that µCT can provide reliable high resolution three dimensional images enabling quantification of the cortical and medullary bone structure.[97]

µCT used to evaluate bone volume around miniscrew implants contains many advantages with some limitations. These advantages include comprehensive evaluation of the bone surrounding the implant surface, non-destructive evaluation of the specimens, and good accuracy with high correlation.[97,98] The largest limitation to the

use of μCT is in its apparent evaluation of actual bone to implant contact. μCT uses ionizing radiation similar to conventional computed tomography scans which creates the possibility for missing data and distortion due to metallic artifact referred to as halation effect or partial volume effect.[99,100] Butz et al performed a study to evaluate accuracy of μCT vs. histology when evaluating bone volume around titanium implants that were 1 mm in diameter and 2 mm in length.[101] There were significant differences in the bone configuration between histologic sections and μCT images in the 0 to 24 μm zone leading them to conclude that μCT was accurate at a distance of 24 to 240 μm. This halation effect and difficulty reading in the 0 to 24 μm zone may be due to the scans being made at a resolution of 8 μm. Miniscrew implant studies performed at Baylor College of Dentistry using μCT to evaluate bone volume around the MSI interface were able to decrease this halation effect and achieve an accurate reading in the 6 to 42 μm zone scanning at a resolution 6 μm.[5,48,78,93]

Histomorphometric Evaluation

Histomorphometric evaluation can be used to quantify and visualize the cellular activity and bone morphology in a two dimensional slice using ground sections. Percentage of bone contact, bone area within the threads, and number of osteocytes can be counted.[102-104] Qualitative and quantitative analysis of bone to implant contact can be done using light microscopy of thin histological sections.[105] Wigianto et al attempted to develop three dimensional models constructed from digitized photographs of a series of two dimensional histological slides to quantify bone to implant contact ratios.[94] Although Wigianto et al were able to construct three dimensional models using serial

two dimensional sections; the models were largely incomplete.[95] While histology has been considered the gold standard for the evaluation of peri-implant contact, the preparation process is time consuming, requires special equipment, expertise, and may cause artifact errors during the grinding procedure.[106]

Bone Cements

Presently, there are no studies evaluating MSIs placed in bone cement. There is however literature in the spine and orthopedic field evaluating surgical screws placed in polymethylmethacrylate or calcium based cements. Most of the spine studies evaluating the effects of cement augmentation on pedicle screw stability indicate that cement augmentation significantly improves the stability.[107-119] Many variables that could affect stability of pedicle screws placed in cement have been evaluated: osteoporosis, reinstrumentation, fenestrated pedicle screws, cement volume, timing after cement injection, and cement type.

Liu et al performed a study on fresh-frozen human cadaveric spines (L1-L4) to compare the stability between conventional pedicle screws (CPS), expansive pedicle screws (EPS), and polymethylmethacrylate-augmented pedicle screws (PS).[107] In the CPS and EPS groups, pilot holes were made and the screws were placed into the hole using a hand driver without any modification. In the PMMA-PS group, the pilot hole was made and 2.5 mL of PMMA was delivered into the pilot hole and the pedicle screw was inserted. Twenty-four hours later, the vertebrae were evaluated under radiographic examination. Following this, axial pullout tests were performed. There was not a significant difference in bone mineral density between the three groups and radiographic

evaluation revealed PMMA surrounding the pedicle screws. The maximum axial pullout strength (F_{max}) for the PMMA-PS and EPS groups were 102.5% and 56.4% greater than the CPS group; respectively, which were statistically significant. The F_{max} for the PMMA-PS group was 29.5% more than the EPS group but that increase was not statistically significant. The energy to failure (E) in the PMMA-PS and EPS groups were 110.2% and 67.3% higher than the CPS group; respectively, which were statistically significant. The E for the PMMA-PS group was 25.6% greater than the EPS group but that increase was also not statistically significant.

Sarzier et al performed a similar study evaluating axial pullout strength (F_{max}) in T12-L5 vertebrae with graded osteoporotic classifications (Grade I to Grade III).[108] They found that the mean increase in F_{max} between pressurized PMMA-PS and CPS groups was 181% for Grade I, 206% for Grade II, and 213% for Grade III osteoporotic spines. They also found that augmentation of osteoporotic vertebrae with PMMA vertebroplasty can significantly increase F_{max} to levels exceeding the strength of the cortical bone.

Zindrick et al evaluated the effect of methyl methacrylate on screws that were reinserted into a stripped screw hole after the previous screw had been loaded to failure.[109] Two groups were tested, 2 mL liquid methyl methacrylate groups either with or without pressure. In the pressurized group, the catheter was inserted until the tip formed a seal against the pedicle wall to allow the cement to be forced into the surrounding medullary bone. They found that methyl methacrylate restored the axial pull out value back to baseline in previously instrumented and stripped holes and

pressurization doubled the original pull out value. A similar study published in 2007 found a 162% increase in pullout strength for revision screws placed with pressurized PMMA compared to those placed without augmentation.[110]

Sven et al found that PMMA augmentation provided less screw displacement in poor bone stock (i.e. osteoporotic vertebrae), whereas they observed no difference in screw migration for normal bone.[111] Zhuang et al found that pedicle screws placed bicortically had similar axial pull out strengths as the unicortical screws placed in PMMA cement in osteoporotic cadaver S1 vertebrae.[112]

Becker et al[113] compared unperforated screws to perforated (fenestrated) screws placed with PMMA and found no difference in the pullout strength as well as epidural leakage of PMMA with the perforated screws; whereas, Chen et al[114] found that solid screws with pre filled cement resulted in significantly higher pullout strength than cemented injection through cannulated screws. Conversely, Chen et al found that PMMA used with cannulated screws significantly increased the pullout strength compared to solid unaugmented pedicle screws.[115] They also found that the amount of cement expressed from the cannulated screws increased with increasing number of radial holes lead to increasing pullout strength for cannulated screws with a larger numbers of radial holes. Interestingly, they also found that tapping pilot holes may decrease the pullout strength of the screws. Kueny et al found that both prefilled and screw injected fenestrated screws exhibited increased pullout strength but the screw injected group had better fatigue resistance. One reason the data for fenestrated screws may be contradictory is that the cement tends to accumulate outside the proximal fenestrations

which may not improve the stability as much as if it were to diffusely cover the screw along the entire tract.[116]

Studies evaluating the effect of cement volume used have contradicting results. Frankel et al[110] and Pare et al[120] found there to be no significant difference in pullout strength with increasing cement volume. However; Burval et al[117], Folsch et al[118], and Chen et al[119] found significantly higher pullout strength with increasing cement volume.

No difference in pullout strength was found for differing insertion times evaluating placement in soft versus further set cement for either PMMA or calcium phosphate cements.[121-123]

McLachlin et al found that the PMMA cement group required more loading cycles for screw loosening than the calcium triglyceride group.[124] Wittenberg et al found a 2.6 fold increase in pullout strength for PMMA and a 2 fold increase in pullout strength for polypropylene glycol-fumarate.[125] Lotz et al found a 68% increase in axial pullout strength for screws placed with carbonated apatite.[126] Moore et al evaluated a revision model where PMMA increased the pullout strength to 147% and calcium phosphate restored the pullout strength back to baseline (102%).[127] Kuhns et al found a 54% increase in force to failure for screws that were pretapped with calium sulfate/calciumphosphate mixture.[128] Renner et al found PMMA to have significantly higher pullout strengths than calium phosphate in both revision and augmentation while both cements restored pullout strength to baseline in revision.[116]

Recently, Wimhurst et al studied the effects of particulate bone cements on the bone-to-implant interface using a rat model.[129] A ceramic pin was inserted into the tibia

of the rats, a control of normal saline and three types of particulate were used from one bone cement base. The cement base: (1) without radio-opacifier, (2) with zirconium dioxide, and (3) with barium sulphate. Fourteen weeks later, the rats were sacrificed and the tibias were processed for histology. The amount of fibrous tissue and/or gap between the bone-to-implant were measured using image analysis. All three types of bone cement were associated with larger areas of bone resorption than the control. The particles of bone cement appeared to cause resorption at the bone-to-implant interface and it was most marked when barium sulfate was used as the radiopaquer.

Primary stability could be enhanced if 1) a material that hardens could be introduced into the trabeculation space and 2) that material adheres to the MSI immediately after insertion. While PMMA accomplishes both of these goals, it is bioinert and has no potential for bone remodeling or osseointegration which is not ideal for MSIs. OsteoCrete is a magnesium based bone cement which can be used as a bone cement or bone filler because it has osteoconductive properties.[11-14] OsteoCrete has been shown to increase the stability of MSIs through its bone to stainless steel adherence properties.[12] Kim et al found that magnesium ion implantation on SLA-treated titanium dental implants demonstrated increased cell attachment and growth, which improved the implants osseointegration capacity.[130] As such, OsteoCrete, a newly developed bone cement that is magnesium based, holds promise for enhancing MSI primary stability. To date, however, the potential use of OsteoCrete with intraorally placed MSIs remains unexplored. Hirvinen et al performed a study evaluating the influence of OsteoCrete on bone-screw interfaces in the third metacarpal and third metatarsal of horses.[12] The use

of Mg-based cement increased the peak torque to failure when compared to both the Ca-based cement and the control group. In this study, the Mg-based cement was not absorbed after 7 weeks. However, a rabbit study found that the Mg-based cement placed in the distal portion of the rabbit femur resulted in 63.6% absorption after 12 weeks and 83.8% absorption after 26 weeks.[a] Schendel et al found that OsteoCrete had a faster resorption and replacement by bone rate than Norian, a calcium based bone cement.[13] They also showed that OsteoCrete produced superior bone flap position and apparent stability. After 24 week, 50% of the OsteoCrete bone cement persisted.

Sehlke et al used four mongrel dogs to place dental implants in extraction sites filled with OsteoCrete and evaluate the biologic response and bone-to-implant contact 4 months later.[131] Mandibular third premolars and first molars were extracted bilaterally and 4.1 mm x 8 mm SLActive Straumann implants were placed in the extraction site where they were supported by only 2 to 3 mm of apical furcation bone. The experimental sites had OsteoCrete placed to fill the extraction defects with the implants placed immediately after and the control sites had the implants placed immediately after the extractions. The dogs were sacrificed 4 months after implant placement and the harvested implant block segments were prepared for undemineralized histologic evaluation under light microscopy. The BIC for the experimental group was 51.7% and the control group was 43.7% which were not statistically significant differences. The implant survival for the experimental group was 6 out of 8 versus 8 out of 8 for the control group. The authors relate this decreased survival rate to the inability to obtain

primary soft tissue closure in some of the implant sites and the effect of the oral cavity on exposed OsteoCrete.

CHAPTER II

INFLUENCE OF SLA SURFACE FINISH ON MSI STABILITY WHEN PLACED ALONG WITH OSTEOCRETE BONE CEMENT

INTRODUCTION

One of the most important considerations in orthodontics is anchorage. While there are many forms of anchorage that orthodontists have at their disposal, miniscrew implants have become an ideal anchorage option as they offer near absolute anchorage and require minimal patient compliance. The main problem with MSIs today is their relatively high failure rates.[1,2] The loss of bone-to-implant contact, which changes during the primary and secondary stability phases has been reported to be the most common cause of MSI failure.[3] Other causes of MSI failure include trauma to the MSI causing bone fracture and pull out. A systematic review reported a failure rate of 16.4% for MSIs.[2] In a retrospective study the MSI failure rate was found to be 11.4%.[1] Failure rates of MSIs are higher than rates of endosseous implants which has been reported in a systematic review to be 5.4% at 10 years.[4] The relatively high failure rates of MSIs contribute to orthodontists avoiding their use. MSI failures often delays treatment, as it requires replacing them in different locations, which is sometimes not available, or placing them in the same location after healing occurs. One of the ways that failure rates could be reduced is by using sandblasted and acid-etched (SLA) MSIs.

SLA treatment increases the area of the implant surface, which results in increased secondary stability because it provides greater bone-to-implant contact.[5] SLA

treated endosseous implants have become the gold standard in dentistry due to the improved healing characteristics associated with them.[6-8] Multiple studies have shown that SLA MSIs have increased removal torque, bone-to-implant contact due to increased osseointegration resulting in higher success rates and increased stability.[5,9,10] SLA MSI stability could be further enhanced by increasing their primary stability.

Primary stability could be enhanced if 1) a material that hardens could be introduced into the trabeculation space and 2) that material adheres to the MSI immediately after insertion. While polymethylmethacrylate (PMMA) accomplishes both of these goals, it is bioinert and has no potential for bone remodeling or osseointegration, which is necessary for the stability of MSIs. OsteoCrete is a newly introduced magnesium based bone cement which can be used as a bone cement or bone filler because it has osteoconductive properties.[11-14] OsteoCrete has been shown to increase the stability of bone screws through its bone-to-stainless steel adherence properties.[12] To date, the potential use of OsteoCrete with intraorally placed MSIs remains unexplored. Hirvinen et al performed a study evaluating the influence of OsteoCrete on bone-screw interfaces in the third metacarpals and third metatarsals of horses.[12] The use of a magnesium-based cement increased the peak torque to failure when compared to both the calcium-based cement and the control group. However, the magnesium-based cement was not resorbed after 7 weeks.

The present study will be the first to evaluate whether increasing the surface area of MSIs placed in OsteoCrete bone cement increases their stability and improves their survival rates. This study will longitudinally compare the stability of SLA finished

MSIs to machine finished MSIs, with both groups of MSIs placed into OsteoCrete. If the working hypothesis is correct (i.e. that the increased surface area and mechanical retention of the SLA finished MSIs placed along with OsteoCrete provides increased primary and secondary stability) it should improve the survival rate of MSIs. This would provide orthodontists a more reliable way to place MSIs and give them more confidence when planning cases that require maximum anchorage.

MATERIALS AND METHODS

This randomized, split mouth design used six skeletally mature male beagle dogs between one to two years of age. The dogs were purchased from Marshall Bioresources (DBA Marshall Farm Group; North Rose, NY). All of the dogs had a full dentition and were healthy before and throughout the project. Dogs were chosen because their bone has been shown to serve as a good model human bone.[132,133] The Institutional Animal Care and Use Committee at Texas A&M University Baylor College of Dentistry approved the care of the dogs and the experimental protocol. The dogs were housed in the Animal Research Unit. All of the dogs underwent a 10 day quarantine period during which they acclimated to the ARU housing and were monitored for weight loss. The study was approved by the Institutional Animal Care and Use Committee at Texas A&M University College of Dentistry (2015-0294-CD).

Miniscrew Design

Two types of MSIs were specifically fabricated for this study. The MSIs were 7 mm in length and 1.6 mm in diameter (Neodent, Curitiba, Parana, Brazil). They were

made of titanium, they were self-drilling, and they had a pitch of 0.7 mm. Both the experimental and control MSIs had threaded SmartPeg Type A3 (Integration Diagnostics, Goteborg, Sweden) accepting heads. The experimental MSIs had their entire threaded surface SLA treated, the collar and head were machine polished. The entire control MSIs had machine polished finishes.

Surgical Procedure

There were 4-5 buccal MSIs placed in each mandibular quadrant. The MSIs were placed interradicularly and interdentally, depending on the space available. On the day of MSI placement, the dogs were weighed and sedated using Ketamine (1.1 - 2.2 mg/kg) and Xylazine (0.11 - 0.22 mg/kg) injected intramuscularly. A prophylaxis was performed using an ultrasonic cavitron (Denstply, York, PA) with a 0.12% chlorhexidine solution in order to decrease the intra-oral bacterial load.[73] The dogs were then intubated and given 1% to 2% isoflurane (Butler Animal Health Supply, Dublin, Ohio) with oxygen at 0.5 - 1 L per minute. Atropine (0.05 mg/kg) (IVX Animal Health) was given subcutaneously to prevent bradycardia. Scout periapical radiographs, taken with a Planmeca Intra X-Ray unit (Planmeca USA, Roselle, IL) and size 4 phosphor plates, were used to determine interradicular and interdental sites with adequate space for MSI placement. Radiographic measurements were transferred intraorally using a periodontal probe. The mandible was anesthetized using 2% lidocaine with 1:100,000 epinephrine via local infiltration with a 27-guage needle. All MSIs were placed in unattached tissue due to the limited amount of attached gingiva available for ideal bone placement (Figure 1). Due to the thickness and density of the cortical bone in the mandible, pilot holes

were pre drilled through the buccal cortex using a 1.1 mm pilot drill (Neodent corporation; Curitiba.PR, Brazil; 3M Corporation; St. Paul, Minnesota, USA) in a slow speed handpiece at 1600 RPMs with copious irrigation. These pilot holes were created in one quadrant at a time to avoid excessive insertion torque and screw fracture. The MSIs were then placed into the pilot holes using a hand driver (Figure 1C) and then backed out to create a hole large enough to allow for the OsteoCrete (Bone Solutions Inc, Colleyville, TX, USA) placement (Figure 1B).

Two mL of injectable OsteoCrete solution was hand mixed for 90 seconds using sterile saline and OsteoCrete bone powder (Figure 1A). This solution was immediately loaded into a 3cc syringe with a thin-walled 18 gauge BD needle. Prior to loading, the needles had been sectioned to a length of 6 mm using a high speed handpiece and a 556 cross cut carbide metal cutting bur. Approximately 0.1 – 0.2 mL of OsteoCrete solution was injected into each of the pilot holes in the quadrant (Figure 1B). Using random assignment, either experimental or control MSIs were then placed using a straight hand driver. They were placed perpendicular to the cortical plate and parallel to the occlusal plane. The screws were inserted until the threads were no longer visible, taking care not to insert into the lingual cortex. Creation of pilot holes, OsteoCrete injection and MSI insertion was then repeated on the opposite mandibular quadrant. Post MSI placement periapical radiographs were taken to verify ideal MSI placement (Figure 2) and intra-oral photos were taken to document tissue appearance. Analgesics (Nalbuphene, 1 - 2 mg/kg SC BID for 3 days then PRN) and antibiotics (Penicillin G Procaine with Benzathine, 20,000 - 40,000 units/kg at the time of surgery) were administered. Eight to

ten MSIs were placed in each dog, for a total of 28 control and 28 experimental MSIs. The dogs body temperature was maintained during surgery using a warm water circulating pad. End tidal carbon dioxide, heart rate, oxygen saturation, respiration rate, and body temperature were monitored.

Inspection of the MSIs, intra-oral photographs, and implant stability quotient measurements were performed weekly for 9 weeks. For the interim weekly measurements, the dogs were sedated with Ketamine (1.1 – 2.2 mg/kg) and Xylazine (0.11 – 0.22 mg/kg) and heart rate, oxygen saturation, respiration rate were monitored. If MSIs were covered by hypertrophic mucosa, the area was anesthetized using 2% Lidocaine with 1:100K epinephrine, and a Vetroson V-10 Bi-Polar Electrosurgical Unit (Summit Hill Laboratories; Navesink, NJ) was used to remove the mucosa overlying the MSI head (Figure 3). During these sedations, heart rate, oxygen saturation, and respiration rate were monitored. In order to evaluate the amount and timing of bony remodeling, each dog underwent IV infusion of Calcein green at 4 and 8 weeks and Alizarin red at 6 weeks for histological fluorescence labeling. All of the fluorescence dyes used were prepared shortly before infusion.

Longitudinal Evaluation

The stability of each MSI was measured using the Osstell Mentor Smartpeg type A3, which measured the implant stability quotient (ISQ). The Osstell transducer was calibrated according to the manufacturer's instructions. The SmartPeg mount was magnetically connected to the SmartPeg type A3, screwed into the head of the MSI, and tightened with finger pressure according to the manufacturer's instructions. The

SmartPeg mount was then removed and the Osstell transducer was oriented perpendicular to the long axis of the SmartPeg type A3/MSI (Figure 4A), and three measurements were recorded for each MSI. To minimize unwanted MSI movement while unscrewing the Smartpeg, the MSI head was secured with a hemostat as the SmartPeg was being removed (Figure 4B). The three measurements were then averaged. Each MSI had 10 implant stability quotient values, including: measurements at day of MSI placement and 9 weekly measurements.

Nine weeks after MSI placement, the dogs were sedated using Ketamine (2.2 mg/kg) and Xylazine (0.22 mg/kg) injected intravenously. Once adequate sedation was confirmed by checking for reflexes, the common carotid arteries were located and cannulated via surgical dissection. The dogs were then euthanized using 2 mL of Beuthanasia-D (Schering Corp, Kenilworth, NJ) given intracardially. Once heart function ceased, the external jugular veins were located and severed to allow for perfusion of 1.5 liters of saline followed by 1 liter of 4% paraformaldehyde (PFA) through the cannulas. The mandible was harvested via en bloc resection using a stryker bone saw and stored in 4% PFA. Each mandibular block was sectioned at the symphysis. The overlying soft tissue was removed using a scalpel handle with a 15 blade and a periosteal elevator. The bone-implant specimens used for the analyses were retrieved using a dremel mounted on a drill press stand with a 10 mm trephine bur (ACE Dental Implant System, Brockton, Mass) under copious irrigation. The specimens were trephined parallel to the long axis of the MSI, ensuring that all 3 layers of the bone

(cortical, medullary, cortical) remained intact and undamaged. The trephined specimens were then stored and labeled in separate jars in 4% PFA.

Data Collection and Analysis

Ten matched (experimental and control) pairs of MSIs were randomly selected for histological evaluations and 15 pairs of MSIs were randomly selected for μCT analysis. Of those selected for histology, three pairs were analyzed using traditional Hematoxylin and Eosin (H&E) and the remaining seven pairs underwent fluorescence and Stevenel's blue stain with Van Giesson picro fuchsin counterstain.

The H&E specimens were fixed in 4% PFA, demineralized in ethylenediaminetetraacetic acid (EDTA), dehydrated in a graded series of ethanol, cleared with xylene, infiltrated and embedded in paraffin. They were sectioned in a horizontal plane at a thickness of 5 to 6 μm. Sectioning was initiated closest to the buccal cortical surface and continued in a lingual direction. Every 15th to 20th section was selected, for a total of 12 sections per sample. The sections were mounted with three per glass slide. They were then stained with hematoxylin and eosin to evaluate the bone appearance and the presence of any inflammatory, osteoclastic, or osteoblastic cells. The H&E images were captured at varying magnifications from 2.5x to 40x using a Zeiss Axioplan microscope (Carl Zeiss Microimaging, Germany) and SPOT 5.0 software (SPOT Imaging Solutions, Sterling Heights, MI) (Figure 5).

The samples selected for the fluorescence group were fixed in 4% PFA, dehydrated in an ascending series of ethanol, and embedded in methyl methacrylate which was allowed to polymerize. The implant block was sectioned from buccal to

lingual, along the horizontal plane, using a Buehler Isomet Low Speed Saw (Buehler Ltd., Lake Bluff, IL, USA). Eight sections were harvested per sample, with each sample being approximately 125 μm thick. The specimens were then hand-ground to a thickness of approximately 100 μm using silicon carbide paper with decreasing coarseness (240, 320, 400, 600 grit) under copious water irrigation. The hand-ground specimens were mounted on coated glass slides and a final polish was completed using number 2 and 3 Buehler micropolishing solutions. The fluorescence images were then acquired 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 a magnification of 5x (Figure 6A and 6B). The fluorescent dies are taken up by the bone when calcium is laid down which provides the ability to visualize bone activity at specific timepoints.

Once all of the fluorescent images were acquired, the fluorescent specimens were stained with Stevenel's Blue and Van Giesson picro fuchsin counterstain for viewing and imaging using a Zeiss Axioplan microscope (Carl Zeiss Microimaging, Germany) and SPOT 5.0 software (SPOT Imaging Solutions, Sterling Heights, MI) at a magnification of 2.5x. This staining procedure viewed under light microscopy allows visualization of the OsteoCrete and bone cells which the confocal imaging does not provide. Each slide was imaged on automatic contrast and also under a higher light intensity (Figure 7A and 7B). It was difficult to visualize the difference between the MSI and OsteoCrete when viewed under automatic brightness; however, the bone staining visualization was ideal under automatic brightness. In order to delineate MSI

from OsteoCrete, the auto brightness was turned off and the light intensity was increased. This allowed for clear distinction between MSI and OsteoCrete as the OsteoCrete had a black to gray granular appearance.

µCT analysis (Figure 8 and 9) was performed using a Bruker's Skyscan 1173 µCT machine with two samples oriented on top of each other in a tube, along with 4% PFA. The specimens were scanned at a resolution of 10 µm. X-ray settings were 130 kVp, 61 µA, and a 1000 ms integration time. The resolution setting of 958 projections per 180° and a 0.25 mm brass filter were used to allow for low metallic halation and high quality scans. The region of interest (ROI) was defined as a cylinder (6000 µm pixel diameter) around the centered MSI. Each scan took an average of 48 minutes per specimen. Threshold limits of, 45-255 (1 gray scale or "brightness" number, above which all voxels will be considered bone, and below which all voxels will be considered non-bone) were determined using ten randomly chosen specimens. The thresholds of 110-255 were used to remove the titanium from the analysis. Datasets were reconstructed and analyzed using Skyscan Nrecon software (Bruker; Kontich, Belgium). The reconstruction settings applied were a Gaussian smoothing of 2, Ring Artifact Correction of 5, Beam Hardening Correction of 20%, and Dynamic Range of [-0.003-0.05].

The ROI started 250 µm apical to the buccal cortical bone and extended 3 mm apically (Figure 9). This was determined using the reconstructed 3-dimensional images. Bone volume fraction (bone volume/total volume) and bone mineral density (gHA/cm^3) was calculated for the ROI. The ROI included one layer of bone, 1 voxel (10

μm) thick, extending 10 to 20 μm from the MSI surface (Figure 9A). The voxel of bone adjacent to the MSI surface (0-10 μm) was excluded because it was subject to metallic halation artifact. 3-D renderings were to show the density and trabeculation of the bone around the MSIs (Figure 9B).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 23.0 software (SPSS Inc.; Chicago, IL) was used for statistical analysis. All measurements were taken by a single investigator and statistical reliability confirmed. A chi-square test was used to determine whether the difference in success rates were significant. The ISQ measurements collected with the Osstell IDx were determined to be normally distributed. A paired t-test was used to determine whether there were differences in weekly ISQ measurements between control and experimental MSIs. Paired t-tests were also used to evaluate whether there were changes over time. The micro CT bone volume fraction and bone mineral density data was not normally distributed. Wilcoxon signed ranks test was run to determine whether the differences between the control and experimental percent bone and bone mineral density were significant. A significance level of $p<0.05$ was used for all of the analyses.

RESULTS

Success Rate

The overall MSI success rate was 96.4% (i.e. failure rate of 3.6%). The MSIs were deemed to be failures if they exhibited gross mobility or were removed during the

careful application and removal of the SmartPeg. Two of the control MSIs failed during the last two weeks of the study, resulting in a success rate of 93.1% (i.e failure rate of 6.9%). The experimental group had no failures, resulting in a 100% success rate. By the second week, approximately one third of the MSIs had developed inflammation and hypertrophic mucosa that required removal (Figure 3). This removal of tissue was required at least every other week throughout the study.

Resonance Frequency Analysis

The ISQ measurements showed statistically significant decreases in both groups through week 4 (Figure 10). The decreases were most pronounced during the first two weeks. The decrease in ISQ values between week 0 to week 2 in the experimental MSIs was significantly greater than the decrease over the same time period in the control MSIs ($p = 0.024$). After week 0, ISQ values were consistently higher in the control than experimental MSIs, with statistically significant ($P<0.05$) differences at weeks 5, 6, 7, and 8.

Micro CT

The median bone volume fraction for the control and experimental MSIs were 72.1 and 74.9%, respectively (Figure 11A). The median bone mineral density for the control and experimental MSIs in the 10-20 μm ROI were 0.960 and 0.986 gHA/cm^3, respectively (Figure 11B). Although the experimental MSIs exhibited slightly greater bone volume fractions and bone mineral densities, than the control MSIs, the differences were not statistically significant ($p>0.05$).

Fluorescence Microscopy

The fluorescent slides viewed under confocal microscopy showed Calcein labeling as a neon green, Alizarin labeling as a neon red, OsteoCrete as a black granular appearance, and MSI as a pale to neon green circle with a thread (Figures 6A and 6B). In the trabecular sections, OsteoCrete was evident in the medullary cavities around the MSI (Figure 6B); however, it was also appreciated in smaller amounts surrounding the MSI in some of the cortical sections (Figure 6A). There was no fluorescent labeling within the OsteoCrete nor along the MSI surface where the OsteoCrete was present. There was however fluorescent labeling around and up to the edge of the OsteoCrete. There was no appreciable difference between control and experimental slides when viewed under fluorescence. There was approximately 50% bone to implant contact for both the control and experimental MSIs.

Stephenel's Blue

The cortical sections showed a thin layer of OsteoCrete surrounding the MSI (Figure 7A and 7B). The medullary sections revealed large amounts of OsteoCrete surrounding the MSI and filling the trabecular spaces at an appreciable distance away from the MSI (Figure 7B). These medullary sections had osteoblasts lining the trabecular spaces in areas where OsteoCrete was not present. The bone adjacent to areas where OsteoCrete had filled the trabecular spaces did not contain osteoblasts but there was bone up to the edge of the OsteoCrete.

Hematoxylin and Eosin

The H&E sections showed general areas of acellular bone adjacent to the area where the MSI and OsteoCrete existed, extending for approximately 0.25 - 0.5 mm (Figure 5). These areas exhibited empty osteocyte lacunae except for bone immediately adjacent to Haversian canals which still contained osteocytes within their lacunae. There were minimal Howship's lacunae and osteoclasts noted, as well as minimal inflammatory cells present. Osteoblasts were generally present in both the cortical and medullary sections indicating normal bone activity. The medullary sections showed what appeared to be remnants of OsteoCrete that may not have been completely demineralized. Aside from these remnants of OsteoCrete it was not possible to determine where the OsteoCrete was located around the MSI because the MSI and demineralized OsteoCrete both appeared as empty voids.

DISCUSSION

The MSIs in the current study had good success rates. The success rate was 93.1% (26/28) for the control MSIs and 100% (28/28) for the experimental MSIs, a difference that was not statistically significant. The overall success rate of 93.1% was similar to values previously reported for dogs.[5,40,53,60] Both failed MSIs developed hypertrophic and inflamed mucosa covering their heads, which required removal of tissue with an electrosurgical unit in order to place the SmartPeg. These failures and tissue reactions were likely due to peri-implant inflammation, which has been reported to be associated with the accumulation of plaque around MSIs.[38,47,55] The pilot holes that

were used could also explain some of the failures. During the initial phase of the present project, it was determined that a 1.1 mm pilot hole was necessary to avoid the high shear forces that resulted in screw fracture. Pilot holes have also been reported to have a negative effect on the stability and success rates of MSIs.[38,48] It has also been reported that MSI success rates may be less ideal when placed in nonkeratinized mucosa, due to increased risk of inflammation and infection.[38,55] All of the MSIs in the present study were placed approximately 1-2 mm apical to the mucogingival junction in nonkeratinized mucosa. Both of the failure sites had large scooped out bony defects where bone had been resorbed, likely due to inflammation around the MSI. With this bony defect the cortical thickness was diminished, which is one of the most important factors in MSI success and stability.[55]

OsteoCrete inhibits the normal healing process that is expected to occur around MSIs. Neither the control nor the experimental MSIs exhibited the expected increase in secondary stability after the third week.[3,5,48] Hodges, who compared control MSIs not placed in OsteoCrete and experimental MSIs placed in OsteoCrete, found normal primary and secondary stability curves for the control MSIs, but not for the experimental MSIs.[134] The experimental MSIs exhibited a stability curve similar to those in the present study. The MSIs in the present study showed decreases in stability from week 0 to week 4, after which stability leveled off through week 9. The decrease in stability from week 0 to week 4 is expected as the damaged bone is removed and remodeled during the primary stability phase.[3,66] MSI stability then leveled off between weeks 4 –

9, indicating that OsteoCrete limited the normal bone healing that occurs at the bone-to-implant interface.

The smooth MSIs exhibited greater stability than the SLA MSIs at all time points, with statistically significant differences at weeks 5, 6, 7, and 8. MSI stability decreased most during the first two weeks, which was also when the group differences were the greatest. It was originally thought that the SLA surface treatment would increase primary stability and enhance mechanical retention if the OsteoCrete flowed onto the roughened surface.[80] SLA treatment increases the surface area of MSIs, resulting in greater rates and degrees of osseointegration, and increased secondary stability because it provides increased bone-to-implant contact.[5,7] However, this did not occur in the present study because the experimental and control MSIs had similar initial ISQ values.

The reason that the experimental MSIs became less stable over time than the smooth MSIs may be related to the inability of the OsteoCrete particles to fill the rough surface of the MSI. This would result in less surface contact than with the smooth surfaced MSIs. It is also possible that the rough surface allows for more bacterial and plaque contamination from the oral cavity than the smooth surface. This would be especially important if the OsteoCrete's expansion and/or micromotions after MSI placement creates a gap between the cement and MSI, or microfractures within the OsteoCrete. The manufacturer reports that OsteoCrete expands 0.15% to 0.2% by volume.[131] Microstructural craze lines were reported in a four month dental implant study that used OsteoCrete as the grafting material around the implants.[131] The

expansion and craze lines may produce gaps between the cement and MSIs, which would make them less stabile and increase the possibility of contamination from the oral cavity. The lack of bone being laid down at the MSI and OsteoCrete interface may explain why secondary stability did not increase and return to baseline in the present study. It appears that the OsteoCrete blocks the fibrin adherence and osteoblastic activity along the MSI interface, acting more as a barrier, rather than a scaffold for osteoinduction and conduction along the MSI.

When the screws are inserted with OsteoCrete, surface treatment has no effect on the amount or strength of bone around the MSIs. Micro CT evaluations did not show differences between the control and experimental MSIs in bone volume fraction or bone mineral density. When inserted without OsteoCrete, increased amounts of bone have been found around SLA treated MSIs when compared to machine polished MSIs.[5] Interestingly, the experimental MSIs in the present study exhibited slightly higher bone volume fraction and bone mineral density. This suggests that bone density is not a good indicator of stability. The OsteoCrete's mineral composition was likely picked up as increased bone mineral.

When bone cement is not involved and only surface characteristics are considered, SLA implants produce greater osseointegration due to the improved healing at the bone to implant interface.[6,51] It is thought that the complex topography of the SLA treatment increases the available surface area for fibrin attachment and entanglement. Entanglement may prevent the detachment of fibrin that occurs during wound healing in

machine polished implants. If the fibrin detaches from the implant surface upon wound contraction, direct synthesis of bone matrix on the implant surface is not able to occur.

OsteoCrete is biocompatible and possibly osteoconductive. There were no inflammatory cells present in any of the histological sections, indicating that the OsteoCrete was not rejected and did not trigger an inflammatory response. OsteoCrete was present in large amounts in the trabecular sections, where it was dispersed into the medullary cavities and generally surrounded the MSI. Smaller amounts of OsteoCrete were surrounding the MSIs in the cortical sections, especially around the experimental MSIs. Fluorescent labeling was observed around and up to the edge of the OsteoCrete, indicative of normal bone activity and suggesting that OsteoCrete may be osteoconductive. Sehlke et al also found that OsteoCrete was inert and did not illicit any type of inflammatory response.[131] They also found bone growth up to and around the OsteoCrete.

While OsteoCrete does not inhibit bone formation, there is no evidence that it was being remodeled after nine weeks. Osteoclasts and cutting cones were not present in any of the histology sections, indicating that OsteoCrete was not actively being resorbed or remodeled. Moreover, fluorescent labeling did not show any activity within the OsteoCrete or near the MSI, indicating that there was no bone activity or mineralization within the OsteoCrete nor at the interface between the MSI and OsteoCrete. Fluorescence was evident at the bone-to-implant contact when OsteoCrete was not present. The bony islands evident within the OsteoCrete were pre-existing. Since the fluorescent images did not show any activity where bony islands were located,

OsteoCrete must have enveloped them as it was forced into the trabecular spaces. It has been previously shown that OsteoCrete does not fully resorb after 4-6 months.[12,131]

Drilling and/or MSI placement causes osteocyte necrosis in the vicinity of the insult. Histology revealed areas of acellular bone adjacent to the MSI and OsteoCrete, extending for approximately 0.5 mm. The areas were void of osteocytes. There were empty osteocyte lacunae, except in areas immediately adjacent to Haversian canals with vascular supply. Acellular areas near traumatic insults have been previously reported.[135,136] The traumatic insult in the present study was caused by heat produced when drilling the pilot hole and/or by microfractures produced during MSI placement. Drilling pilot holes at low speed has been shown to produce heat above 47°C, which can result in bone necrosis.[71,137,138] High levels of strain in cortical bone during MSI placement have also been shown to cause microdamage in bone that extends well beyond the implant surface.[139-141] However, this was at least potentially mitigated by the pilot holes, which have been shown to cause less bone displacement and strain when MSIs are inserted.[139]

Other cements have been shown to improve screw stability. Polymethylmethacrylate (PMMA) and calcium based cements improve screw stability during orthopedic procedures.[107-119] However, the improvements in screw stability that orthopedics have been able to achieve may not be transferrable to orthodontic MSIs for two reasons. First, orthopedic screws are placed in a sterile environment, while orthodontic MSIs are placed in the oral cavity, where there is a direct communication between the oral flora and the MSI. Second, the gold standard cement in orthopedics is

PMMA, which bonds to the screw and balloons into the medullary space to increase retention and stability. OsteoCrete does not actually bond to the titanium surface of the MSI and there is slight expansion. The problem with PMMA is that it does not resorb and could interfere with orthodontic movement.

Limitations

The OsteoCrete used in the current study was relatively thick in relation to injecting it through the thin walled 18 gauge BD needle. This was the smallest needle that could be used that allowed the OsteoCrete to flow through. This required creating a 1.1 mm pilot hole in order to fit the needle and deliver the OsteoCrete using thumb pressure.

Some of the MSIs were covered with mucosa at the weekly measurements requiring removal in order to access the head to screw the SmartPeg in. A Vetroson V-10 Bi-Polar Electrosurgical Unit was used to remove the mucosa overlying these MSI heads. It is possible that this surgical insult resulted in inflammation and outer cortical bone resorption resulting in decreased cortical thickness for these MSIs. This should have affected both groups equally as both the control and experimental MSIs had similar numbers of MSIs requiring tissue removal.

There is not a way to determine the exact reason the SLA MSIs had lower stability measurements than the machine polished MSIs. The interface between the MSI and OsteoCrete could not be precisely visualized without the halation effect of the micro CT and the histological processing required to prepare the slides.

Clinical Implications

Although OsteoCrete has been shown to remodel and resorb, it does not resorb as quickly as desired for bone healing to occur around the screw. This prevents secondary stability from occurring and results in less stability than expected. The OsteoCrete formulation used in the current study does not have ideal properties for use in orthodontics with MSIs. The composition of OsteoCrete is monopotassium phosphate (54%), magnesium oxide (41%), tricalcium phosphate (8%), monosodium phosphate (3%), and dextrose (4%). It does not contain any type of osteoclastic recruiting or inducing substances. Osteopontin has been shown to play a crucial role in the formation, adhesion, and function of osteoclasts.[142-144] Substance P activates NF-kappaB and directly facilitates RANKL-induced macrophage osteoclastogenesis and bone resorption activity.[145] Gelatin sponges have also been used as carriers for recombinant human bone morphogenetic protein-2 (rhBMP-2) to increase calcium content and cell growth.[146] If such substances could be incorporated into OsteoCrete, it would speed up the resorption time and enhance bone cell recruitment, resulting in a more efficient bone cement.

The SLA treatment did not result in increased stability when used with OsteoCrete. SLA MSIs appear to provide increased stability when used alone; however, based on the results in the current study it is not recommended to use SLA MSIs with OsteoCrete.[5,7,9,51] If the OsteoCrete properties could be improved, such as finer particle size and the ability to bond to titanium, it could increase the stability when used with SLA MSIs.

CHAPTER III

CONCLUSIONS

The following conclusions were drawn from the study:

1. The MSIs had good success rates with 96.4% remaining stable throughout the 9 week study period.
2. Smooth MSIs exhibited greater stability than the SLA MSIs due primarily to a greater decrease in stability during the first two weeks.
3. For MSIs inserted with OsteoCrete, surface treatment had no effect on the amount or strength of bone around the MSIs.
4. OsteoCrete is biocompatible and possibly osteoconductive, but there was no evidence it was being remodeled after nine weeks.
5. Osteocyte necrosis was evident in the vicinity of the insult.

REFERENCES

1. Kuroda S, Sugawara Y, Deguchi T, Kyung H-M, Takano-Yamamoto T. Clinical use of miniscrew implants as orthodontic anchorage: success rates and postoperative discomfort. American Journal of Orthodontics and Dentofacial Orthopedics 2007;131:9-15.

2. Schätzle M, Männchen R, Zwahlen M, Lang NP. Survival and failure rates of orthodontic temporary anchorage devices: a systematic review. Clinical Oral Implants Research 2009;20:1351-1359.

3. Ure DS, Oliver DR, Kim KB, Melo AC, Buschang PH. Stability changes of miniscrew implants over time. The Angle Orthodontist 2011;81:994-1000.

4. Moraschini V, Poubel LAdC, Ferreira VF, Barboza EdSP. Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. International Journal of Oral and Maxillofacial Surgery 2015;44:377-388.

5. Ikeda H, Rossouw PE, Campbell PM, Kontogiorgos E, Buschang PH. Three-dimensional analysis of peri-bone-implant contact of rough-surface miniscrew implants. American Journal of Orthodontics and Dentofacial Orthopedics 2011;139:e153-e163.
6. Davies JE. Mechanisms of endosseous integration. Int J Prosthodont 1998;11:391-401.

7. Abrahamsson I, Berglundh T, Linder E, Lang NP, Lindhe J. Early bone formation adjacent to rough and turned endosseous implant surfaces. Clinical Oral Implants Research 2004;15:381-392.

8. Franchi M, Bacchelli B, De Pasquale V, Martini D, Ruggeri A, Giavaresi G et al. Influence of different implant surfaces on peri-implant osteogenesis: Histomorphometric analysis in sheep. Journal of Periodontology 2007;78:879-888.

9. Chang CS, Lee TM, Chang CH, Liu JK. The effect of microrough surface treatment on miniscrews used as orthodontic anchors. Clinical Oral Implants Research 2009;20:1178-1184.

10. Kim SH, Lee SJ, Cho IS, Kim SK, Kim TW. Rotational resistance of surface-treated mini-implants. Angle Orthodontist 2009;79:899-907.

11. Gulotta LV, Kovacevic D, Ying L, Ehteshami JR, Montgomery S, Rodeo SA. Augmentation of tendon-to-bone healing with a magnesium-based bone adhesive. The American Journal of Sports Medicine 2008;36:1290-1297.

12. Hirvinen LJM, Litsky AS, Samii VF, Weisbrode SE, Bertone AL. Influence of bone cements on bone-screw interfaces in the third metacarpal and third metatarsal bones of horses. American Journal of Veterinary Research 2009;70:964-972.

13. Schendel SA, Peauroi J. Magnesium-based bone cement and bone void filler: preliminary experimental studies. The Journal of Craniofacial Surgery 2009;20:461-464.

14. Waselau M, Samii VF, Weisbrode SE, Litsky AS, Bertone AL. Effects of a magnesium adhesive cement on bone stability and healing following a metatarsal osteotomy in horses. American Journal of Veterinary Research 2007;68:370-378.

15. Gainsforth BL, Higley LB. A study of orthodontic anchorage possibilities in basal bone. American Journal of Orthodontics and Oral Surgery 1945;31:406-417.

16. Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scandinavian Journal of Plastic and Reconstructive Surgery 1969;3:81-100.

17. Brånemark PI. Osseointegration and its experimental background. The Journal of Prosthetic Dentistry 1983;50:399-410.

18. Roberts WE, Helm FR, Marshall KJ, Gongloff RK. Rigid endosseous implants for orthodontic and orthopedic anchorage. Angle Orthodontist 1989;59:247-256.

19. Saito S, Sugimoto N, Morohashi T, Ozeki M, Kurabayashi H, Shimizu H et al. Endosseous titanium implants as anchors for mesiodistal tooth movement in the beagle dog. American Journal of Orthodontics and Dentofacial Orthopedics 2000;118:601-607.

20. Higuchi KW, Slack JM. The use of titanium fixtures for intraoral anchorage to facilitate orthodontic tooth movement. The International Journal of Oral & Maxillofacial Implants 1991;6:338-344.

21. Kokich VG. Managing complex orthodontic problems: the use of implants for anchorage. Seminars in Orthodontics 1996;2:153-160.

22. Odman J, Lekholm U, Jemt T, Brånemark PI, Thilander B. Osseointegrated titanium implants--a new approach in orthodontic treatment. European Journal of Orthodontics 1988;10:98-105.

23. Roberts WE, Smith RK, Zilberman Y, Mozsary PG, Smith RS. Osseous adaptation to continuous loading of rigid endosseous implants. American Journal of Orthodontics 1984;86:95-111.

24. Smalley WM, Shapiro PA, Hohl TH, Kokich VG, Brånemark PI. Osseointegrated titanium implants for maxillofacial protraction in monkeys. American Journal of Orthodontics and Dentofacial Orthopedics 1988;94:285-295.

25. Van Roekel NB. Use of Brånemark system implants for orthodontic anchorage: report of a case. The International Journal of Oral & Maxillofacial Implants 1989;4:341-344.

26. Turley PK, Kean C, Schur J, Stefanac J, Gray J, Hennes J et al. Orthodontic force application to titanium endosseous implants. Angle Orthodontist 1988;58:151-162.

27. Kanomi R. Mini-implant for orthodontic anchorage. Journal of Clinical Orthodontics: JCO 1997;31:763-767.

28. Costa A, Raffainl M, Melsen B. Miniscrews as orthodontic anchorage: a preliminary report. The International Journal of Adult Orthodontics and Orthognathic Surgery 1998;13:201-209.

29. Creekmore TD, Eklund MK. The possibility of skeletal anchorage. Journal of Clinical Orthodontics: JCO 1983;17:266-269.

30. Saito S, Ohmae M, Morohashi T, Seki K, Qu H, Kanomi R et al. A clinical and histological evaluation of titanium mini-implants as anchors for orthodontic intrusion in the beagle dog. American Journal of Orthodontics and Dentofacial Orthopedics 2001;119:489-497.

31. Park HS, Bae SM, Kyung HM, Sung JH. Micro-implant anchorage for treatment of skeletal Class I bialveolar protrusion. Journal of Clinical Orthodontics : JCO 2001;35:417-422.

32. Lee JS, Park HS, Kyung HM. Micro-implant anchorage for lingual treatment of a skeletal Class II malocclusion. Journal of Clinical Orthodontics : JCO 2001;35:643-647; quiz 620.

33. Bae SM, Park HS, Kyung HM, Kwon OW, Sung JH. Clinical application of micro-implant anchorage. Journal of Clinical Orthodontics : JCO 2002;36:298-302.

34. Park HS, Kyung HM, Sung JH. A simple method of molar uprighting with micro-implant anchorage. Journal of Clinical Orthodontics : JCO 2002;36:592-596.

35. Kyung HM, Park HS, Bae SM, Sung JH, Kim IB. Development of orthodontic micro-implants for intraoral anchorage. Journal of Clinical Orthodontics : JCO 2003;37:321-328; quiz 314.

36. Chen ST, Ivanovski S, Nixon KC. A retrospective analysis of 1000 consecutively placed implants in private practice. Australian Dental Journal 2009;54:123-129.

37. Buser D, Janner SFM, Wittneben J-G, Brägger U, Ramseier CA, Salvi GE. 10-year survival and success rates of 511 titanium implants with a sandblasted and acid-etched surface: a retrospective study in 303 partially edentulous patients. Clinical Implant Dentistry and Related Research 2012;14:839-851.

38. Cheng S, Tseng I, Lee J, Kok S. A prospective study of the risk factors associated with failure of mini-implants used for orthodontic anchorage. International Journal of Oral & Maxillofacial Implants 2004;19:100-106 107p.

39. Chaddad K, Ferreira AFH, Geurs N, Reddy MS. Influence of surface characteristics on survival rates of mini-implants. The Angle Orthodontist 2008;78:107-113.

40. Woods PW, Buschang PH, Owens SE, Rossouw PE, Opperman LA. The effect of force, timing, and location on bone-to-implant contact of miniscrew implants. European Journal of Orthodontics 2009;31:232-240.

41. Crismani AG, Bertl MH, Celar AG, Bantleon H-P, Burstone CJ. Miniscrews in orthodontic treatment: review and analysis of published clinical trials. American Journal of Orthodontics and Dentofacial Orthopedics 2010;137:108-113.

42. Reynders R, Ronchi L, Bipat S. Mini-implants in orthodontics: A systematic review of the literature. American Journal of Orthodontics & Dentofacial Orthopedics 2009;135:564.e561-564.e519.

43. Ashley ET, Covington LL, Bishop BG, Breault LG. Ailing and failing endosseous dental implants: a literature review. The Journal of Contemporary Dental Practice 2003;4:35-50.

44. Park HS, HwangBo ES, Kwon TG. Proper mesiodistal angles for microimplant placement assessed with 3-dimensional computed tomography images. American Journal of Orthodontics and Dentofacial Orthopedics 2010;137:200-206.

45. Heidemann W, Gerlach KL, Gröbel KH, Köllner HG. Influence of different pilot hole sizes on torque measurements and pullout analysis of osteosynthesis screws. Journal of Cranio-Maxillo-Facial Surgery 1998;26:50-55.

46. Gantous A, Phillips JH. The effects of varying pilot hole size on the holding power of miniscrews and microscrews. Plastic and Reconstructive Surgery 1995;95:1165-1169.

47. Park H, Jeong S, Kwon O. Factors affecting the clinical success of screw implants use as orthodontic anchorage. American Journal of Orthodontics & Dentofacial Orthopedics 2006;130:18-111 110p.

48. Carney LO, Campbell PM, Spears R, Ceen RF, Melo AC, Buschang PH. Effects of pilot holes on longitudinal miniscrew stability and bony adaptation. American Journal of Orthodontics and Dentofacial Orthopedics 2014;146:554-564.

49. Büchter A, Koerdt S, Wiesmann HP, Piffko J, Meyer U, Wiechmann D. Load-related implant reaction of mini-implants used for orthodontic anchorage. Clinical Oral Implants Research 2005;16:473-479.

50. Buschang PH, Carrillo R, Ozenbaugh B, Rossouw PE. 2008 survey of AAO members on miniscrew usage. Journal of Clinical Orthodontics : JCO 2008;42:513-518.

51. Davies JE. Understanding peri-implant endosseous healing. Journal of Dental Education 2003;67:932-949.

52. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. Clinical Oral Implants Research 2003;14:251-262.

53. Owens SE, Buschang PH, Cope JB, Franco PF, Rossouw PE. Experimental evaluation of tooth movement in the beagle dog with the mini-screw implant for orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2007;132:639-646.

54. Wilmes B, Rademacher C, Olthoff G, Drescher D. Parameters affecting primary stability of orthodontic mini-implants. Journal of Orofacial Orthopedics 2006;67:162-174.

55. Miyawaki S, Koyama I, Inoue M, Mishima K, Sugahara T, Takano-Yamamoto T. Factors associated with the stability of titanium screws placed in the posterior region for orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2003;124:373-378.

56. Huja SS, Litsky AS, Beck FM, Johnson KA, Larsen PE. Pull-out strength of monocortical screws placed in the maxillae and mandibles of dogs. American Journal of Orthodontics and Dentofacial Orthopedics 2005;127:307-313.

57. Motoyoshi M, Hirabayashi M, Uemura M, Shimizu N. Recommended placement torque when tightening an orthodontic mini-implant. Clinical Oral Implants Research 2006;17:109-114.

58. Lim HJ, Eun CS, Cho JH, Lee KH, Hwang HS. Factors associated with initial stability of miniscrews for orthodontic treatment. American Journal of Orthodontics & Dentofacial Orthopedics 2009;136:236-242 237p.

59. Motoyoshi M, Yoshida T, Ono A, Shimizu N. Effect of cortical bone thickness and implant placement torque on stability of orthodontic mini-implants. The International Journal of Oral & Maxillofacial Implants 2007;22:779-784.

60. Salmória KK, Tanaka OM, Guariza-Filho O, Camargo ES, de Souza LT, Maruo H. Insertional torque and axial pull-out strength of mini-implants in mandibles of dogs. American Journal of Orthodontics and Dentofacial Orthopedics 2008;133:790.e715-722.

61. Cleek TM, Reynolds KJ, Hearn TC. Effect of screw torque level on cortical bone pullout strength. Journal of Orthopaedic Trauma 2007;21:117-123.

62. Huja SS, Rao J, Struckhoff JA, Beck FM, Litsky AS. Biomechanical and histomorphometric analyses of monocortical screws at placement and 6 weeks postinsertion. The Journal of Oral Implantology 2006;32:110-116.

63. Cope JB. OrthoTADs : the clinical guide and atlas. Dallas, Tex. : Under Dog Media, 2006.

64. Piattelli A, Trisi P, Romasco N, Emanuelli M. Histologic analysis of a screw implant retrieved from man: influence of early loading and primary stability. The Journal of Oral Implantology 1993;19:303-306.

65. Chen Y, Shin H-I, Kyung H-M. Biomechanical and histological comparison of self-drilling and self-tapping orthodontic microimplants in dogs. American Journal of Orthodontics and Dentofacial Orthopedics 2008;133:44-50.

66. Kohn D, Rose C. Primary stability of interference screw fixation. American Journal of Sports Medicine 1994;22:34.

67. Hung E, Oliver D, Kim KB, Kyung H-M, Buschang PH. Effects of pilot hole size and bone density on miniscrew implants' stability. Clinical Implant Dentistry and Related Research 2012;14:454-460.

68. Ansell RH, Scales JT. A study of some factors which affect the strength of screws and their insertion and holding power in bone. Journal of Biomechanics 1968;1:279-302.

69. Lim SM, Hong RK. Distal movement of maxillary molars using a lever-arm and mini-implant system. Angle Orthodontist 2008;78:167-175.

70. Brinley CL, Behrents R, Kim KB, Condoor S, Kyung H-M, Buschang PH. Pitch and longitudinal fluting effects on the primary stability of miniscrew implants. The Angle Orthodontist 2009;79:1156-1161.

71. Eriksson AR, Albrektsson T. Temperature threshold levels for heat-induced bone tissue injury: a vital-microscopic study in the rabbit. The Journal of Prosthetic Dentistry 1983;50:101-107.

72. Buschang PH, Carrillo R, Rossouw PE. Orthopedic correction of growing hyperdivergent, retrognathic patients with miniscrew implants. Journal of Oral and Maxillofacial Surgery: Official Journal of The American Association of Oral and Maxillofacial Surgeons 2011;69:754-762.

73. Carrillo R, Buschang PH. Palatal and mandibular miniscrew implant placement techniques. Journal of Clinical Orthodontics: JCO 2013;47:737-743.

74. Cochran DL. A comparison of endosseous dental implant surfaces. Journal of Periodontology 1999;70:1523-1539.

75. Romanos G, Toh CG, Siar CH, Swaminathan D, Ong AH, Donath K et al. Peri-implant bone reactions to immediately loaded implants. An experimental study in monkeys. Journal of Periodontology 2001;72:506-511.

76. Nkenke E, Lehner B, Weinzierl K, Thams U, Neugebauer J, Steveling H et al. Bone contact, growth, and density around immediately loaded implants in the mandible of mini pigs. Clinical Oral Implants Research 2003;14:312-321.

77. Mortensen MG, Buschang PH, Oliver DR, Kyung H-M, Behrents RG. Stability of immediately loaded 3- and 6-mm miniscrew implants in beagle dogs--a pilot study. American Journal of Orthodontics and Dentofacial Orthopedics 2009;136:251-259.

78. Massey CC, Kontogiorgos E, Taylor R, Opperman L, Dechow P, Buschang PH. Effect of force on alveolar bone surrounding miniscrew implants: a 3-dimensional microcomputed tomography study. American Journal of Orthodontics and Dentofacial Orthopedics 2012;142:32-44.

79. Park J-C, Kim H-D, Kim S-M, Kim M-J, Lee J-H. A comparison of implant stability quotients measured using magnetic resonance frequency analysis from two directions: a prospective clinical study during the initial healing period. Clinical Oral Implants Research 2010;21:591-597.

80. Javed F, Ahmed HB, Crespi R, Romanos GE. Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation. Interventional Medicine & Applied Science 2013;5:162-167.

81. Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J. Long-standing plaque and gingivitis at implants and teeth in the dog. Clinical Oral Implants Research 1992;3:99-103.

82. Trisi P, Rebaudi A. Progressive bone adaptation of titanium implants during and after orthodontic load in humans. International Journal of Periodontics & Restorative Dentistry 2002;22:30-43 14p.

83. Reyes CD, Petrie TA, Burns KL, García AJ, Schwartz Z. Biomolecular surface coating to enhance orthopaedic tissue healing and integration. Biomaterials 2007;28:3228-3235.

84. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. Journal of Biomedical Materials Research 1991;25:889-902.

85. Wennerberg A, Albrektsson T, Andersson B, Krol JJ. A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. Clinical Oral Implants Research 1995;6:24-30.

86. Orton EC, Polher O, Shenk R, Hohn RB. Comparison of porous titanium-surfaced and standard smooth-surfaced bone plates and screws in an unstable fracture model in dogs. American Journal of Veterinary Research 1986;47:677-682.

87. Meredith N, Alleyne D, Cawley P. Quantitative determination of the stability of the implant-tissue interface using resonance frequency analysis. Clinical Oral Implants Research 1996;7:261-267.

88. Sennerby L, Meredith N. Implant stability measurements using resonance frequency analysis: Biological and biomechanical aspects and clinical implications. Periodontology 2000 2008;47:51-66.

89. Aparicio C, Lang NP, Rangert B. Validity and clinical significance of biomechanical testing of implant/bone interface. Clinical Oral Implants Research 2006;17 Suppl 2:2-7.

90. Pattijn V, Jaecques SVN, Muraru L, Van der Perre G, Vander Sloten J, De Smet E et al. Resonance frequency analysis of implants in the guinea pig model: Influence of boundary conditions and orientation of the transducer. Medical Engineering and Physics 2007;29:182-190.

91. Zix J, Hug S, Kessler-Liechti G, Mericske-Stern R. Measurement of dental implant stability by resonance frequency analysis and damping capacity assessment: Comparison of both techniques in a clinical trial. International Journal of Oral and Maxillofacial Implants 2008;23:525-530.

92. Glauser R, Rée A, Hämmerle CHF, Sennerby L, Lundgren A, Gottlow J et al. Resonance frequency analysis of implants subjected to immediate or early functional occlusal loading. Successful vs. failing implants. Clinical Oral Implants Research 2004;15:428-434.

93. Cuairán C, Campbell PM, Kontogiorgos E, Taylor RW, Melo AC, Buschang PH. Local application of zoledronate enhances miniscrew implant stability in dogs. American Journal of Orthodontics and Dentofacial Orthopedics 2014;145:737-749.

94. Wigianto R, Ichikawa T, Kanitani H, Horiuchi M, Matsumoto N, Ishizuka H. Three-dimensional examination of bone structure around hydroxyapatite implants using digital image processing. Journal of Biomedical Materials Research 1997;34:177-182.

95. Van Oossterwyck H, Duyck J, Vander Sloten J, Van der Perre G, Jansen J, Wevers M et al. Use of microfocus computerized tomography as a new technique for characterizing bone tissue around oral implants. The Journal of Oral Implantology 2000;26:5-12.

96. Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M. The direct examination of three-dimensional bone architecture in vitro by computed tomography. Journal of Bone and Mineral Research 1989;4:3-11.

97. Müller R, Van Campenhout H, Van Der Perre G, Van Damme B, Dequeker J, Hildebrand T et al. Morphometric analysis of human bone biopsies: A quantitative structural comparison of histological sections and micro-computed tomography. Bone 1998;23:59-66.

98. Kuhn JL, Goldstein SA, Feldkamp LA, Goulet RW, Jesion G. Evaluation of a microcomputed tomography system to study trabecular bone structure. Journal of Orthopaedic Research 1990;8:833-842.

99. Zhao S, Robertson DD, Wang G, Whiting B, Bae KT. X-ray CT metal artifact reduction using wavelets: An application for imaging total hip prostheses. IEEE Transactions on Medical Imaging 2000;19:1238-1247.

100. Glover GH, Pelc NJ. Nonlinear partial volume artifacts in x-ray computed tomography. Medical Physics 1980;7:238-248.

101. Butz F, Ogawa T, Chang TL, Nishimura I. Three-dimensional bone-implant integration profiling using micro-computed tomography. International Journal of Oral and Maxillofacial Implants 2006;21:687-695.

102. Sennerby L, Thomsen P, Ericson LE. A morphometric and biomechanic comparison of titanium implants inserted in rabbit cortical and cancellous bone. The International Journal of Oral & Maxillofacial Implants 1992;7:62-71.

103. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: a 1-year follow-up of removal torque of titanium implants. The International Journal of Oral & Maxillofacial Implants 1987;2:69-75.

104. Ericsson I, Johansson CB, Bystedt H, Norton MR. A histomorphometric evaluation of bone-to-implant contact on machine-prepared and roughened titanium dental implants. A pilot study in the dog. Clinical Oral Implants Research 1994;5:202-206.

105. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. Journal of Oral Pathology 1982;11:318-326.

106. Kihara A, Morimoto K, Suetsugu T. Improved method using a bubble-free adhesion technique for the preparation of semi-serial undecalcified histologic sections containing dental implants. The Journal of Oral Implantology 1989;15:87-94.

107. Liu D, Wu ZX, Fu SC, Gao MX, Shi L, Lei W et al. Biomechanical comparison of different techniques in primary spinal surgery in osteoporotic cadaveric lumbar vertebrae: Expansive pedicle screw versus polymethylmethacrylate-augmented pedicle screw. Archives of Orthopaedic and Trauma Surgery 2011;131:1227-1232.

108. Sarzier JS, Evans AJ, Cahill DW. Increased pedicle screw pullout strength with vertebroplasty augmentation in osteoporotic spines. Journal of Neurosurgery 2002;96:309-312.

109. Zindrick MR, Wiltse LL, Widell EH, Thomas JC, Holland WR, Field BT et al. A biomechanical study of intrapeduncular screw fixation in the lumbosacral spine. Clinical Orthopaedics and Related Research 1986;NO. 203:99-112.

110. Frankel BM, D'Agostino S, Wang C. A biomechanical cadaveric analysis of polymethylmethacrylate-augmented pedicle screw fixation. Journal of Neurosurgery: Spine 2007;7:47-53.

111. Sven H, Lorin B, Yannick L, Daniel B, Paul H. Influence of screw augmentation in posterior dynamic and rigid stabilization systems in osteoporotic lumbar vertebrae: A biomechanical cadaveric study. Spine 2014;39:E384-E389.

112. Zhuang XM, Yu BS, Zheng ZM, Zhang JF, Lu WW. Effect of the degree of osteoporosis on the biomechanical anchoring strength of the sacral pedicle screws: An in vitro comparison between unaugmented bicortical screws and polymethylmethacrylate augmented unicortical screws. Spine 2010;35:E925-E931.

113. Becker S, Chavanne A, Ogon M, Aigner N, Spitaler R, Kropik K et al. Assessment of different screw augmentation techniques and screw designs in osteoporotic spines. European Spine Journal 2008;17:1462-1469.

114. Chen L-H, Tai C-L, Lee D-M, Lai P-L, Lee Y-C, Niu C-C et al. Pullout strength of pedicle screws with cement augmentation in severe osteoporosis: a comparative study between cannulated screws with cement injection and solid screws with cement pre-filling. BMC Musculoskeletal Disorders 2011;12:33-33.

115. Chen LH, Tai CL, Lai PL, Tsai TT, Fu TS, Niu CC et al. Pullout strength for cannulated pedicle screws with bone cement augmentation in severely osteoporotic bone: Influences of radial hole and pilot hole tapping. Clinical Biomechanics 2009;24:613-618.

116. Renner SM, Lim TH, Kim WJ, Katolik L, An HS, Andersson GB. Augmentation of pedicle screw fixation strength using an injectable calcium phosphate cement as a function of injection timing and method. Spine 2004;29:E212-216.

117. Burval DJ, McLain RF, Milks R, Inceoglu S. Primary pedicle screw augmentation in osteoporotic lumbar vertebrae: Biomechanical analysis of pedicle fixation strength. Spine 2007;32:1077-1083.

118. Fölsch C, Goost H, Figiel J, Paletta JRJ, Schultz W, Lakemeier S. Correlation of pull-out strength of cement-augmented pedicle screws with CT-volumetric measurement of cement. Biomedizinische Technik. Biomedical Engineering 2012;57:473-480.

119. Chen Y-L, Chen W-C, Chou C-W, Chen J-W, Chang C-M, Lai Y-S et al. Biomechanical study of expandable pedicle screw fixation in severe osteoporotic bone comparing with conventional and cement-augmented pedicle screws. Medical Engineering & Physics 2014;36:1416-1420.

120. Paré PE, Chappuis JL, Rampersaud R, Agarwala AO, Perra JH, Erkan S et al. Biomechanical evaluation of a novel fenestrated pedicle screw augmented with bone cement in osteoporotic spines. Spine 2011;36:E1210-E1214.

121. Masaki T, Sasao Y, Miura T, Torii Y, Kojima A, Aoki H et al. An experimental study on initial fixation strength in transpedicular screwing augmented with calcium phosphate cement. Spine 2009;34:E724-E728.

122. Linhardt O, Lüring C, Matussek J, Hamberger C, Grifka J, Herold T et al. Stability of anterior vertebral body screws after kyphoplasty augmentation: An experimental study to compare anterior vertebral body screw fixation in soft and cured kyphoplasty cement. International Orthopaedics 2006;30:366-370.

123. Cho W, Wu C, Erkan S, Mehbod AA, Transfeldt EE, Kang MM. The effect on the pullout strength by the timing of pedicle screw insertion after calcium phosphate cement injection. Journal of Spinal Disorders and Techniques 2011;24:116-120.

124. McLachlin SD, Dunning CE, Saleh KA, Gurr KR, Bailey SI, Bailey CS. Comparative assessment of sacral screw loosening augmented with PMMA versus a calcium triglyceride bone cement. Spine 2011;36:E699-E704.

125. Wittenberg RH, Lee KS, Shea M, White Iii AA, Hayes WC. Effect of screw diameter, insertion technique, and bone cement augmentation of pedicular screw fixation strength. Clinical Orthopaedics and Related Research 1993:278-287.

126. Lotz JC, Hu SS, Chiu DFM, Yu M, Colliou O, Poser RD. Carbonated apatite cement augmentation of pedicle screw fixation in the lumbar spine. Spine 1997;22:2716-2723.

127. Moore DC, Maitra RS, Farjo LA, Graziano GP, Goldstein SA. Restoration of pedicle screw fixation with an in situ setting calcium phosphate cement. Spine 1997;22:1696-1705.

128. Kuhns CA, Reiter M, Pfeiffer F, Choma TJ. Surgical strategies to improve fixation in the osteoporotic spine: the effects of tapping, cement augmentation, and screw trajectory. Global Spine Journal 2014;4:47-54.

129. Wimhurst JA, Brooks RA, Rushton N. The effects of particulate bone cements at the bone-implant interface. Journal of Bone and Joint Surgery - Series B 2001;83:588-592.

130. Kim BS, Kim JS, Park YM, Choi BY, Lee J. Mg ion implantation on SLA-treated titanium surface and its effects on the behavior of mesenchymal stem cell. Mater Sci Eng C Mater Biol Appl 2013;33:1554-1560.

131. Sehlke BM, Wilson TG, Jones AA, Yamashita M, Cochran DL. The use of a magnesium-based bone cement to secure immediate dental implants. The International Journal of Oral & Maxillofacial Implants 2013;28:e357-e367.

132. Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 1998;139:663-670.

133. Wang X, Mabrey JD, Agrawal CM. An interspecies comparison of bone fracture properties. Bio-Medical Materials & Engineering 1998;8:1.

134. Hodges P. Evaluation of Mini-screw Implant Stability with OsteoCrete Bone Cement Orthodontics: Texas A&M University College of Dentistry; 2017.

135. Gemert LV. Localization of the osseous boundaries of the regional acceleratory phenomenon Orthodontics: Texas A&M University College of Dentistry; 2016.

136. Sugay C. The Effect of Increased Cortical Damage on Bone Density and Bone Turnover in Mature Rabbits: A Randomized Split-Body Study Orthodontics: Texas A&M University College of Dentistry; 2017.

137. Chacon GE, Bower DL, Larsen PE, McGlumphy EA, Beck FM. Heat production by 3 implant drill systems after repeated drilling and sterilization. Journal of Oral & Maxillofacial Surgery (02782391) 2006;64:265-269.

138. Eriksson AR, Albrektsson T, Albrektsson B. Heat caused by drilling cortical bone: Temperature measured in vivo in patients and animals. Acta Orthopaedica 1984;55:629-631.

139. Nam O, Yu W, Kyung HM. Cortical bone strain during the placement of orthodontic microimplant studied by 3D finite element analysis. Korean Journal of Orthodontics 2008;38:228-239.

140. Park JS, Yu W, Kyung HM, Kwon OW. Finite element analysis of cortical bone strain induced by self-drilling placement of orthodontic microimplant. Korean Journal of Orthodontics 2009;39:203-212.

141. Wawrzinek C, Sommer T, Fischer-Brandies H. Microdamage in cortical bone due to the overtightening of orthodontic microscrews. Journal of Orofacial Orthopedics 2008;69:121-134.

142. Oh Y, Oh I, Morimoto J, Uede T, Morimoto A. Osteopontin has a crucial role in osteoclast-like multinucleated giant cell formation. Journal of Cellular Biochemistry 2014;115:585-595.

143. Standal T, Borset M, Sundan A. Role of osteopontin in adhesion, migration, cell survival and bone remodeling. Experimental Oncology 2004;26:179-184.

144. Chellaiah MA, Biswas R, Kizer N, Alvarez U, Strauss-Schoenberger J, Hruska KA et al. Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. Molecular Biology of the Cell 2003;14:173-189.

145. Wang L, Zhao R, Shi X, Wei T, Halloran BP, Clark DJ et al. Substance P stimulates bone marrow stromal cell osteogenic activity, osteoclast differentiation, and resorption activity in vitro. Bone 2009;45:309-320.

146. Yokota S, Sonohara S, Yoshida M, Murai M, Shimokawa S, Fujimoto R et al. A new recombinant human bone morphogenetic protein-2 carrier for bone regeneration. International Journal of Pharmaceutics 2001;223:69-79.

APPENDIX A

FIGURES

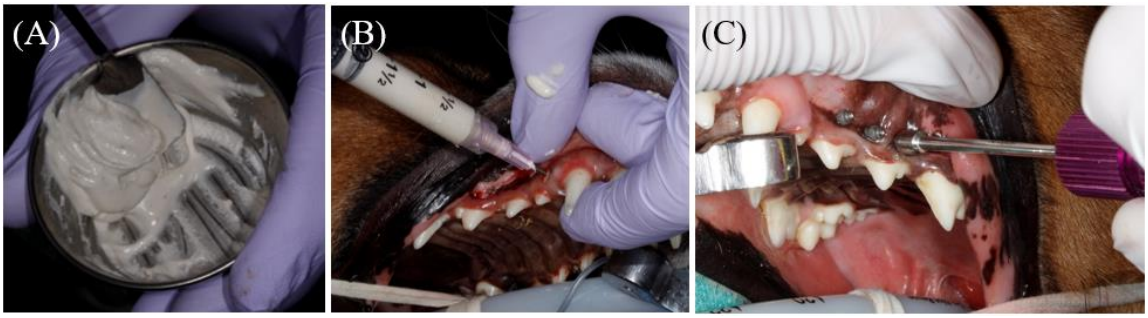

Figure 1. (A) Mixing OsteoCrete (B) Injecting OsteoCrete (C) Placing MSIs

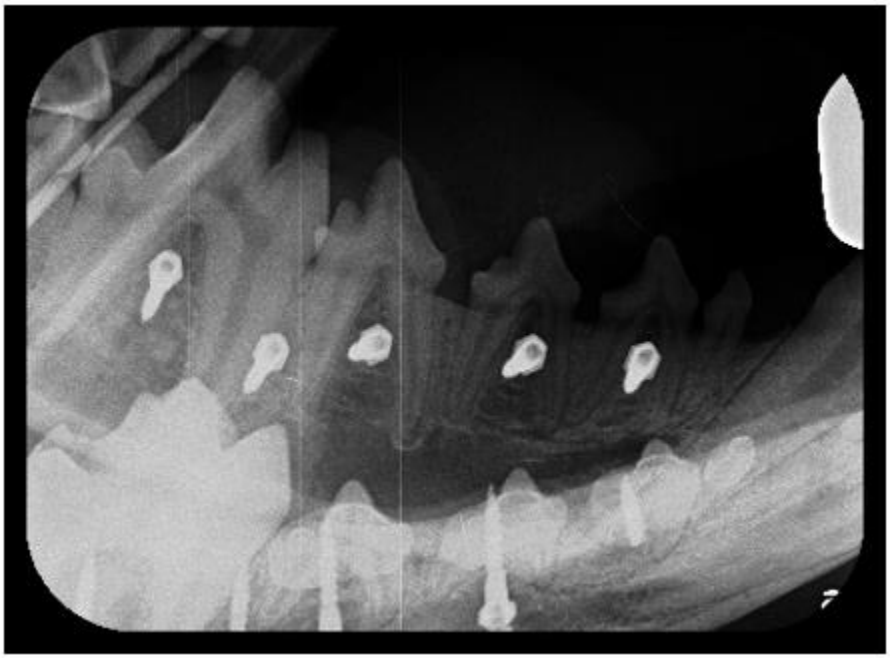

Figure 2. Initial MSI Placement Radiographs.

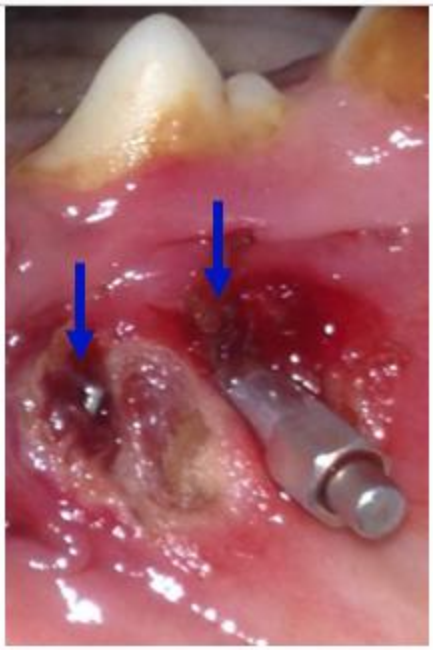

Figure 3. Inflammation and Mucosal Overgrowth. There was inflammation and mucosal overgrowth around the MSIs placed in the mandibular PM4-M1 and M1 locations which required electrosurgical removal for SmartPeg placement. Seen here is tissue removed that was overlying two MSIs (Blue arrows).

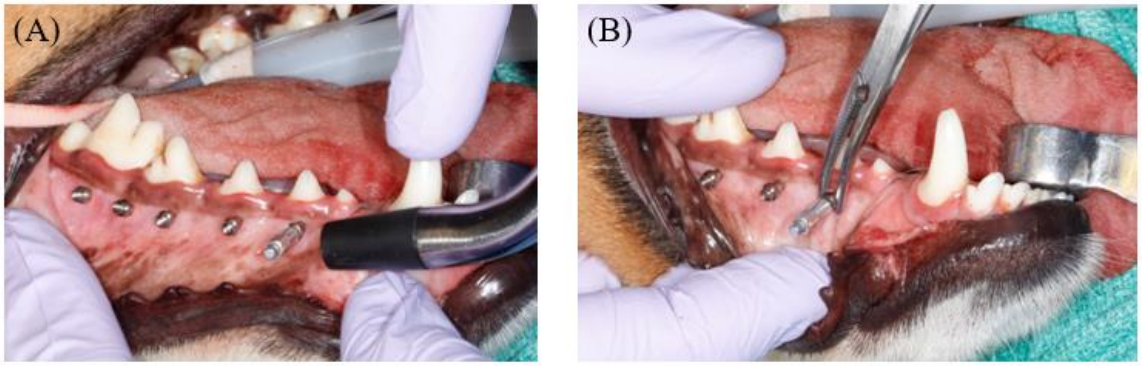

Figure 4. SmartPeg Type A3 Placement. (A) was screwed into the head of the MSIs using forefinger and thumb with a hemostat to stabilize the MSIs. (B) Osstell Idx transducer used to record ISQ measurements.

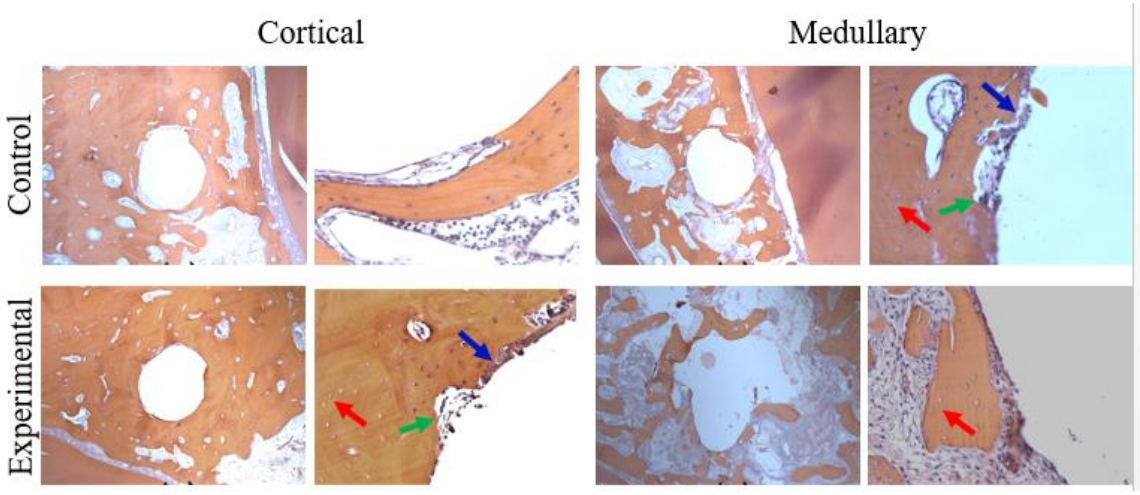

Figure 5. Hematoxylin and Eosin Histological Images. Green arrow = Howship's lacunae with osteoclasts, red arrow = empty lacunae, blue arrow = osteoblasts. There is no evidence of inflammatory cells present with normal osteoblastic activity noted.

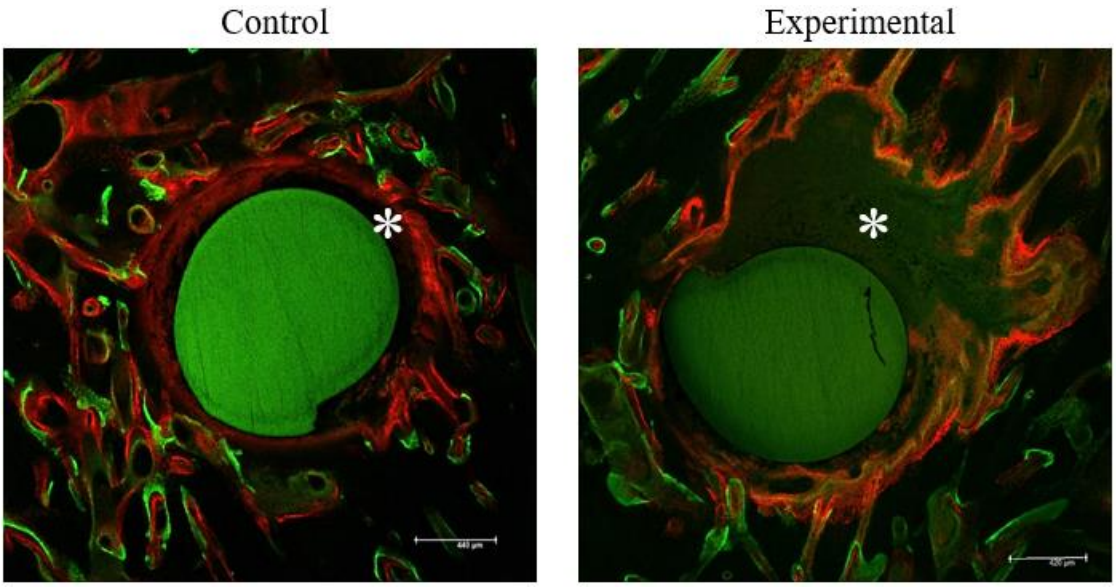

Figure 6A. Confocal Fluorescent Histological Images of the Cortical Layers. MSI centered with OsteoCrete around the MSIs (OsteoCrete = *).

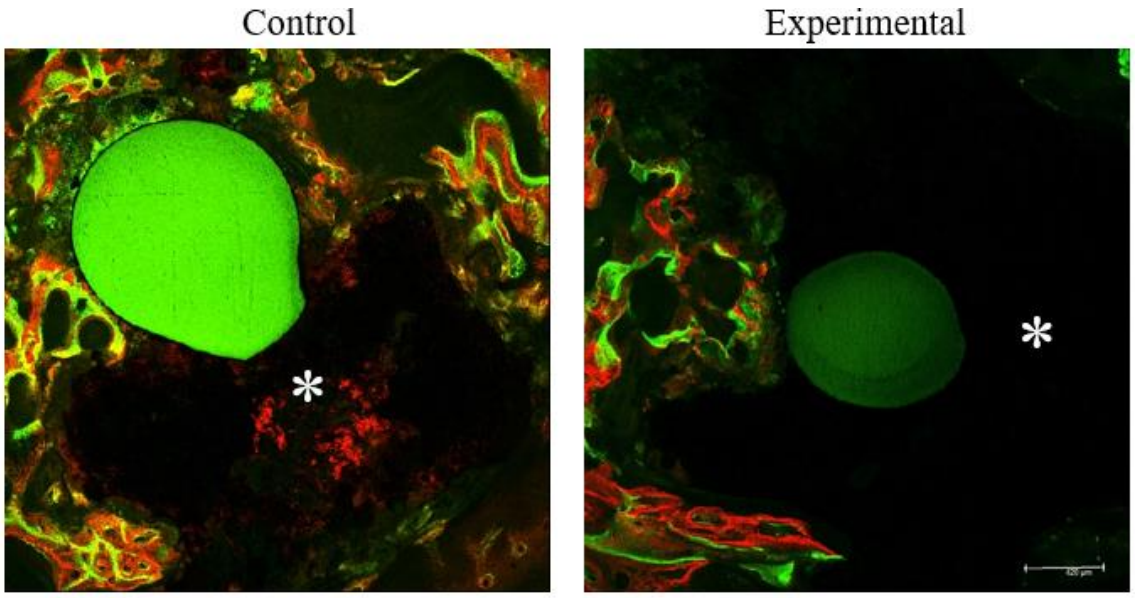

Figure 6B. Confocal Fluorescent Histological Images of the Medullary Layers. MSI centered with large amounts of OsteoCrete around the MSIs (OsteoCrete = *).

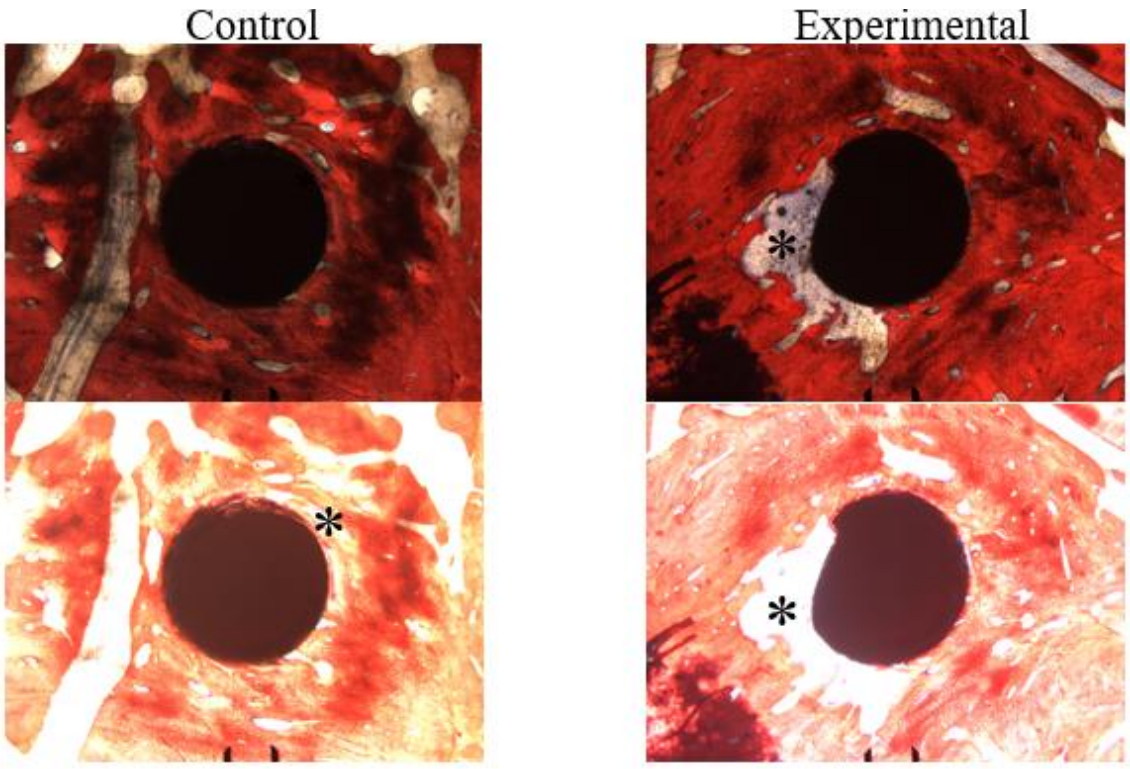

Figure 7A. Cortical Sections: Stevenel's Blue Histological Images. OsteoCrete = *

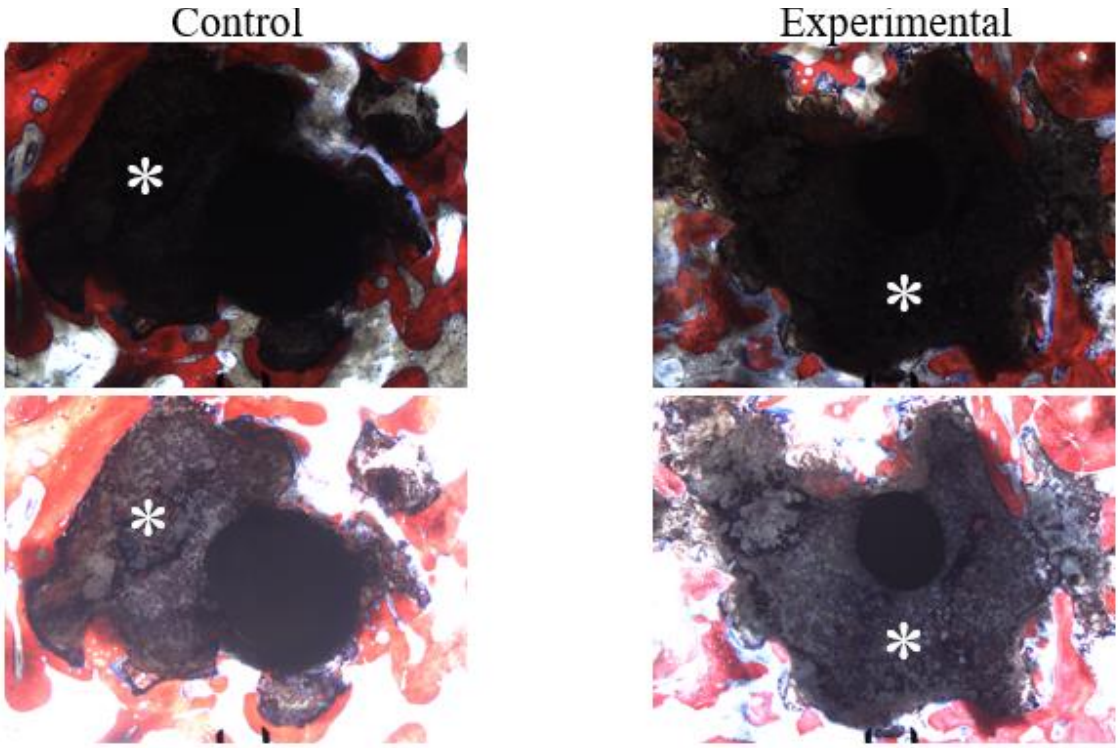

Figure 7B. Medullary Sections: Stevenel's Blue Histological Images. OsteoCrete = *

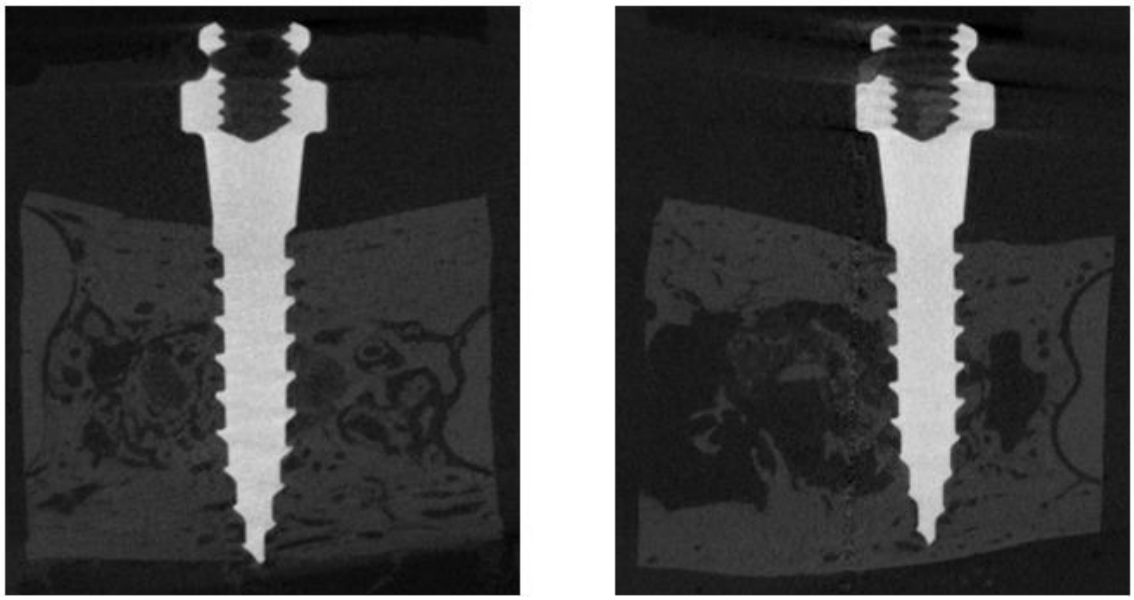

Figure 8. µCT Analysis. Original gray-scale 2D cross section image showing MSI in center (white), surrounding bone (gray), and space (black). The buccal cortical bone is the side towards the head of the MSIs.

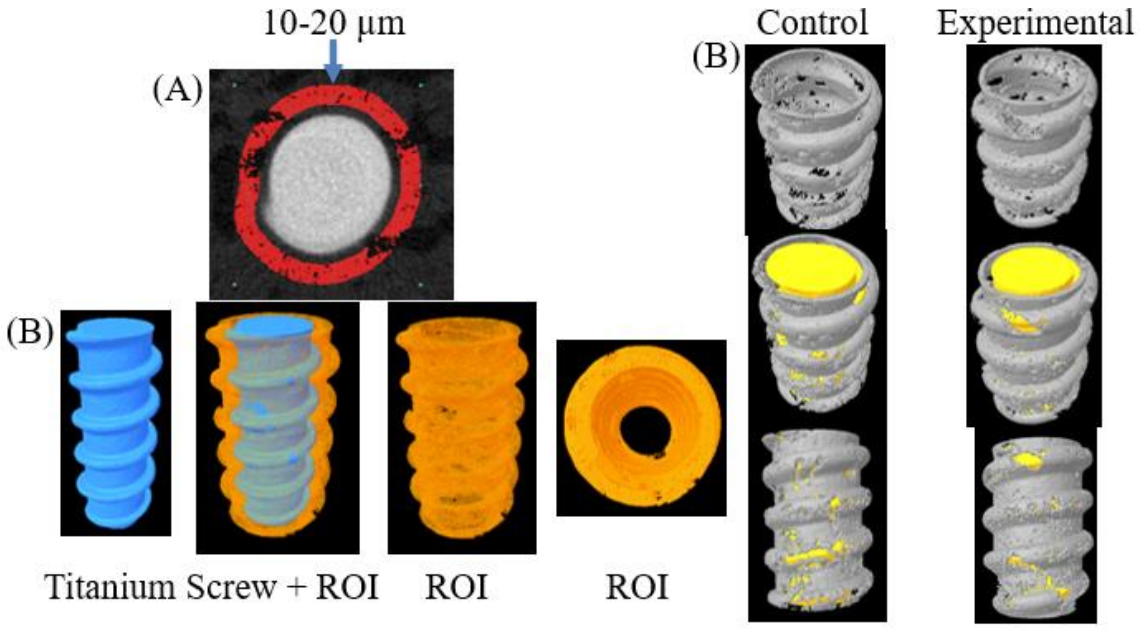

Figure 9. µCT 3D Renderings & ROI. (A) 10-20 µm ROI. (B) MSIs were segmented out and 3D ROI remained.

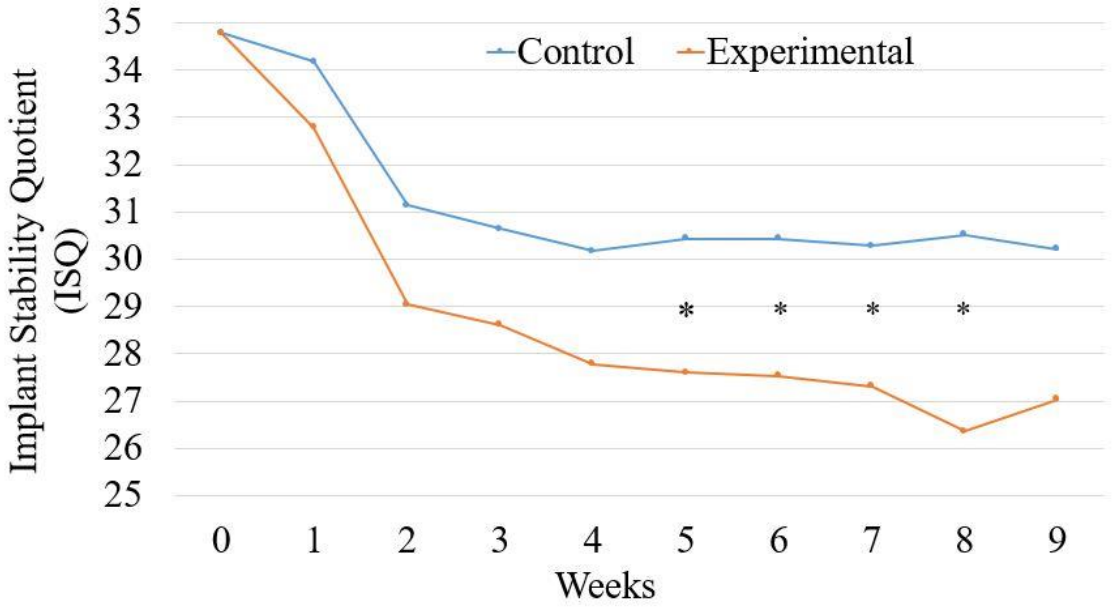

Figure 10. Longitudinal ISQ Measurements of Control (smooth) vs Experimental (SLA) MSIs over the 9 Week Experiment (* = $p<0.05$).

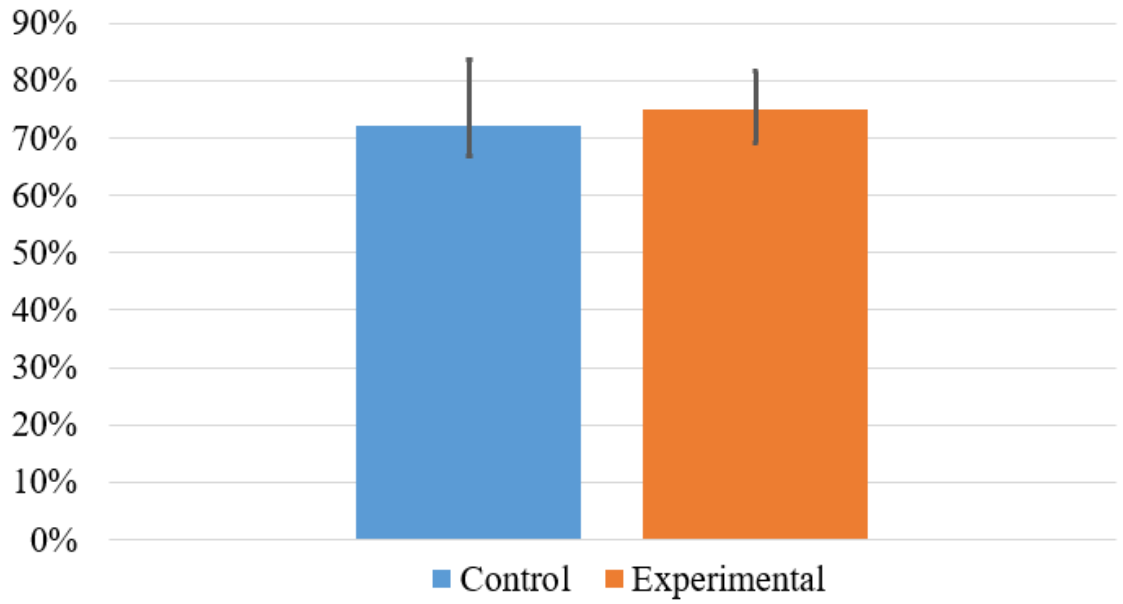

Figure 11A. 10-20 µm – Bone Volume Fractions (BV/TV).

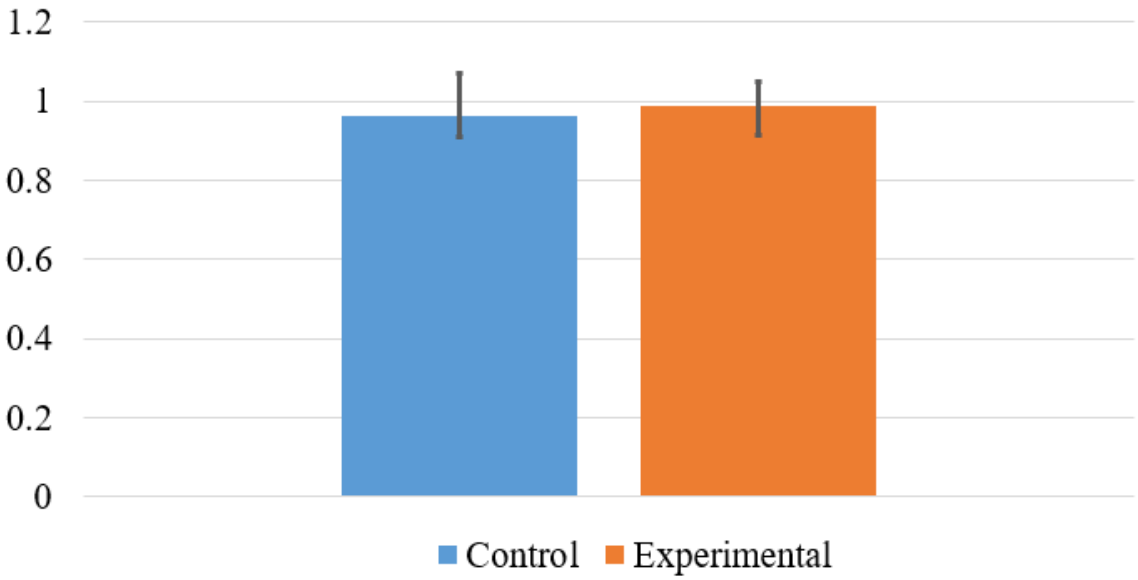

Figure 11B. 10-20 µm – Bone Mineral Density (gHA/cm^3).

APPENDIX B

TABLES

Table 1. Chi-Square Test for Success Rates.

	Success	Failure	
Control	26	2	Chi-square statistic = 2.0741
Experimental	28	0	p-value = 0.15

Table 2. Osstell IDx Implant Stability Quotient (ISQs) Statistics. Mean weekly ISQ values for the control and experimental MSIs with paired samples T-test.

	Control		Experimental		Side Differences	
Week	Mean	SD	Mean	SD	Mean	P-value
0	34.63	7.81	34.89	8.12	-0.26	0.753
1	33.93	8.92	32.89	7.66	1.04	0.288
2	30.93	9.10	29.07	7.84	1.85	0.094
3	30.48	9.00	28.56	6.74	1.93	0.114
4	30.11	8.45	27.70	7.25	2.41	0.087
5	30.37	9.64	27.52	6.72	2.85	0.026
6	30.44	9.61	27.37	6.87	3.07	0.030
7	30.30	10.25	27.15	6.87	3.15	0.048
8	30.54	10.06	26.38	7.21	4.15	0.015
9	30.23	11.48	27.12	7.21	3.12	0.084