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Evaluation of plasma sprayed hydroxyapatite coated AO cortical screws in equine third metacarpal bone

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EVALUATION OF PLASMA SPRAYED HYDROXYAPATITE COATED AO CORTICAL
SCREWS IN EQUINE THIRD METACARPAL BONE

A Thesis

Submitted to the Graduate School of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Master of Sciences

in

The Interdepartmental Program in Veterinary Medical Sciences
through the Department of Veterinary Clinical Sciences

by
Myra E. Durham
DVM, North Carolina State University, 2007
August 2012

DEDICATION

I would like to dedicate this to my parents Larry and Dianne Durham and to my fiancé John Ladner for their continuous support and encouragement.

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I would like to thank the members of my graduate committee, Gary A. Sod, Laura M. Riggs, and Colin F. Mitchell for their support, guidance and patience throughout the project and while I completed this thesis, as well as for the clinical training they've provided throughout my residency program. This project would not have been possible without their help, and I sincerely appreciate all that they have done.

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ABSTRACT

Objectives - To compare the osteointegration of plasma sprayed hydroxyapatite (HA) coated and uncoated 5.5 mm cortical screws in equine third metacarpal (MC3) bones in combination with a 4.5mm broad dynamic compression plate (DCP).

Study design - In vivo study.

Animals – 6 Adult Thoroughbred horses.

Methods – For each horse, four HA coated screws were placed unicortically through a 4.5mm broad DCP in the dorsal cortex of a randomly chosen MC3 bone, with 4 uncoated screws placed in an identical manner in the contralateral MC3 bone. All screws were tightened to a torque of 5.4 N-m. Extraction torques were recorded for the screws when removed after 12 weeks.

The mean extraction torques for HA coated and uncoated screws were compared to insertion torque using a paired *t*-test with the statistical significance set at $p < 0.05$.

Results – Mean extraction torque of the HA coated screws was significantly ($p < 0.0002$) greater than 5.4 N-m. The mean extraction torque of the uncoated screws was significantly ($p < 0.0001$) less than 5.4 N-m. The mean extraction torque for the HA coated screws were significantly ($p < 0.0001$) greater than that of the uncoated screws.

Conclusion - The results suggest that osteointegration was occurring for the HA coated screws. The results also suggest that osteointegration did not take place during the 12 weeks period for the uncoated screws.

Clinical Relevance - The use of plasma sprayed HA coated screws may provide a means of improving the stability of internal fixation of an equine long bone fractures during the entire healing period by promoting osteointegration.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Structure of Bone

Bone is a composite of both organic and inorganic components. It consists of approximately 25% water, 30% organic material, and 40% inorganic components by volume (Markel and Lopez, 2012). The inorganic component is mostly crystalline hydroxyapatite and 90% of the organic component is type I collagen. At the most basic level of bone hydroxyapatite crystals are embedded between the ends of adjoining collagen fibrils (Tencer and Johnson, 1994). In normal bone, the inorganic components provide stiffness and strength and the collagen provides ductility, which is the ability to plastically deform without fracture and the ability to absorb energy (Markel and Lopez, 2012). By combining the rigid characteristics of hydroxyapatite with the flexible nature of collagen, bone is more ductile than hydroxyapatite and more rigid than collagen. This allows bone to absorb more energy before failing and allows for greater load bearing and stiffness than either of these individual components could. As the inorganic matrix matures its solubility decreases and mineralization increases making the bone stiffer (Nunamaker, 2002). The modulus of elasticity (stiffness) of cortical bone increases as the fourth power of the calcium content. Calcium content also affects fatigue life with decreasing calcium levels giving longer fatigue life. Equine cortical bone has a modulus of 18-20 giga pascals (Gpa) (Nunamaker, 2002).

Osteoblasts, osteoclasts, and osteocytes are the predominant cell type in bone (Markel and Lopez, 2012). Osteoblasts develop through an undifferentiated lineage from stem cells. They are found on the surfaces of bone and produce organic matrix to form new bone (Nunamaker, 2002). Osteoblasts also regulate mineralization by releasing small membrane-bound matrix vesicles that concentrate calcium and phosphate and enzymatically destroy mineralization inhibitors (Markel

and Lopez, 2012). Osteoclasts are derived from mononuclear precursor cells of the monocyte/macrophage line and originate in the bone marrow. They secrete multiple enzymes that are responsible for digesting the organic matrix (Markel and Lopez, 2012). Osteoclasts form saucer shaped Howship's lacunae on the surface of cancellous bone and form the front of the "cutting cone" in cortical bone. These cells are mobile and able to respond to the need for local resorption of bone (Nunamaker, 2002). Osteocytes are formed when osteoblasts become imbedded in their own matrix production. These cells have extensive cytoplasmic processes that connect them to bone surface lining cells, osteoblasts, and other osteocytes (Markel and Lopez, 2012). This network of cells covers over 90% of the mature bone matrix (Nunamaker, 2002). Osteocytes are linked through gap junctions which are required for their maturation, activity, and survival (Markel and Lopez, 2012). When bone formation is complete, 50% to 70% of osteoblasts undergo apoptosis with the remainder becoming osteocytes or bone-lining cells. These bone-lining cells control mineral ion influx and efflux of the bone extracellular fluid and they also retain the ability to differentiate back into osteoblasts.

Bone serves to provide the structural framework for the body as a whole. Muscles attach to this frame and this allows for movement. It also affords protection to vital internal organs as well as serving as a site for red blood cell production. Bone is often perceived as an inert structure, however, this is inaccurate. Bone is highly adaptive and is unique in its ability to remodel after injury without scar formation (Markel and Lopez, 2012).

Bones can be divided into three major categories: flat, cuboidal, and long. Flat bones surround and protect vital structures; cuboidal bones make up complex joints, while long bones make up the majority of both the appendicular and peripheral skeleton (Markel and Lopez, 2012). Long bones can be divided into an epiphysis at either end which has an articular surface, a long,

narrower portion in the center which is called the diaphysis, and a metaphysis on either end which is the transition from diaphysis to epiphysis. All skeletal segments have an outer cortex of compact (cortical) bone and an inner medulla containing bone marrow as well as spongy (cancellous) bone (Markel and Lopez, 2012). The ratio of cortex to medulla varies with the function of the bone. Cortical thickness decreases towards the metaphysis and cuboidal bones also have thinner cortices. Both cortical and cancellous bone are composed of osteons, with cortical osteons called Haversian systems and cancellous osteons called packets (Markel and Lopez, 2012). Cortical bone has an apparent density of 1.85 g/cm³. Cancellous bone is less dense with an apparent density of 0.9 g/cm³ or less (Nunamaker, 2002). Cortical bone may have a porosity of only 5%, whereas cancellous bone may be greater than 20% (Nunamaker 2002).

Periosteum is a layer of osteogenic and fibroblastic cells, which are within a nerve and microvascular network, and lies along the periosteal cortex of cortical bone (Markel and Lopez, 2012). It is made up of two distinct layers. The outer fibrous layer contains fibroblasts, collagen, elastin, and a nerve and microvascular network. The inner cambium layer contains adult mesenchymal progenitor cells, differentiated osteogenic progenitor cells, osteoblasts, fibroblasts, microvessels, and sympathetic nerves (Markel and Lopez, 2012). This provides the cells for fracture healing and appositional bone growth. Endosteum is a membranous structure that covers the inner surface of cortical and cancellous bone as well as the blood vessel canals (Volkman's canals). It contains blood vessels, osteoblasts, and osteoclasts (Markel and Lopez, 2012).

Blood supply of bone is provided through both a medullary and periosteal route. The outer one third of cortical bone is supplied by the periosteum and the inner two thirds are supplied by the medullary source (Nunamaker, 2002). The afferent vascular system contains nutrient arteries, proximal and distal metaphyseal arteries, and periosteal arterioles (Markel and

Lopez, 2012). The efferent vascular system contains the large emissary and nutrient veins that drain the medullary cavity, cortical channels, and periosteal capillaries. Periosteal capillaries are connected to those of the cortex, but under normal conditions no blood passes centripetally from periosteum to cortex because of the centrifugal pressure gradient across the cortical capillaries in adult animals (Markel and Lopez, 2012). Primary blood supply and direction of blood flow in the afferent vascular system changes during long bone growth. The periosteal contribution is greater in the younger animal than at skeletal maturity.

1.2 Fracture biology

The mechanical properties of bone depend on the direction of applied forces, which is known as anisotropy. Materials that exhibit neither structural orientation nor dependency on loading orientation are said to be isotropic (Markel and Lopez, 2012). Bone is strongest in compression, weaker in shear, and weakest in tension. Bending forces produce tension on the convex surface of the bone, and torsion forces will resolve into tension forces as well so bones are also weak in both bending and torsion (Nunamaker, 2002). Bending forces also produce compression on the concave surface of the bone. Cortical bone is more brittle than cancellous bone and fails at a lower strain. Cancellous bone stores more energy prior to failure when compared to cortical bone because it is less brittle (Markel and Lopez, 2012). Loading of bone determines its shape: functional requirements may lead bone to model and remodel to change its shape and internal architecture. Bone overload may create injury and fracture (Nunamaker, 2002).

Fatigue failure of bone can occur when bone is repeatedly loaded below its breaking strength. Deformation and strain response of bone to load depends on bone anatomy and

composition as well as direction, rate, magnitude, frequency, and duration of applied load (Markel and Lopez, 2012). Normal daily activity will subject the bone to complex loading conditions and bone's response to loading depends on the rate and application of load. Fatigue load can cause progressive accumulation of microdamage in cortical bone and as it continues the bone will fail through coalescence and propagation of the cracks. An example of this is seen in Thoroughbred race horses in training that develop dorsal metacarpal disease and stress fractures (Nunamaker, 2002).

As a bone deforms it stores the applied energy as strain, and this stored energy is released when a bone fractures or when load is removed. Bones loaded rapidly fail at a higher load and release more energy than those loaded slowly (Markel and Lopez, 2012). Thus, fractures that occur with rapid loading have more comminution and soft tissue injury than those at lower loading rates. This is seen in racehorses experiencing proximal phalangeal, proximal sesamoid, or third metacarpal/tarsal condylar fractures due to high strain and rapid loading repetitive motion fatigue.

Fracture healing involves a series of complex biological events controlled by intracellular and extracellular signaling over a set time frame (Markel and Lopez, 2012). Fracture healing can be divided into direct (primary) and indirect (secondary) bone healing. Primary healing occurs when there is adequate reduction of the fracture fragments and rigid internal fixation that minimizes interfragmentary strain. This process allows for direct regeneration of the Haversian system and occurs by direct growth of secondary osteons from one fragment to another. There is no net resorption of bone in this type of healing and it is characterized by no callus formation at the site of fracture. Secondary healing is what most often occurs in equine fracture healing. With secondary healing the bone heals through the process of endochondral bone formation because the fracture fragments are not stable enough or there is inadequate reduction of fracture fragments

to allow for primary bone healing. Secondary bone healing is characterized by the formation of a callus that remodels over time.

There are three stages of fracture healing: inflammatory, repair, and remodeling. During the inflammatory stage damaged osteocytes release enzymes that break down the organic matrix and this necrotic material induces an inflammatory response (Markel and Lopez, 2012). Initially, a hematoma forms and this is followed by the development of an early callus. The initial callus is soft and contains mostly type III collagen. The repair phase overlaps the inflammatory phase while the majority of the callus is unmineralized. As this matrix becomes mineralized it forms woven bone in which the collagen fibrils are randomly distributed. Bone formation continues in the callus by both intramembranous and endochondral ossification and this is dependent on the amount of instability present. Formation of a bony bridging callus is the final step in the repair phase and this can take anywhere from 2-12 months to occur. The remodeling phase is the final and longest in fracture repair. During this stage, woven bone is remodeling into lamellar bone. The end product of bone healing is regenerated bone that is able to withstand physiologic stresses and strains normally. Stress is force per unit area applied to the bone and strain is the resulting response of the bone to this stress which results in deformation.

1.3 AO/ASIF and Equine Fracture Repair

The Arbeitsgemeinschaft für Osteosynthesefragen (Association for the Study of Internal Fixation) was formed in 1958 to research the concepts of immediate functional rehabilitation after rigid internal fixation of fractures. Not only have they researched osteosynthesis, they have also developed instruments and implants that promote rigid internal fixation. This has evolved into a worldwide organization for the development of orthopedic implants in both the human and veterinary medical field (Colton, 1981).

Compression in rigid fixation has been recognized as an important component of fracture healing. Internal fixation allows for a faster return to function for the patient. This allows movement of joints which promotes joint health by nourishing the articular cartilage and preventing proteoglycan loss (Markel and Lopez, 2012). In addition to these benefits, disuse osteopenia and tendon laxity or contracture can be avoided as there is no longer a need for long term casting of the fractured limb. Equine patients also bring the additional burden of generally weighing more than a thousand pounds and require immediate full weight bearing on the affected limb to minimize the risk of support limb laminitis (Nunamaker, 2002).

When repairing fractures with internal fixation the surgical approach can either be a traditional open approach or more recently via a minimally invasive technique. With the minimally invasive techniques, fracture fragments are reduced and implants are placed through stab incisions. The biggest advantage to a minimally invasive approach is that the vascular integrity of the fracture is not further compromised by the disruption of the surrounding soft tissues. Also, incisions into badly bruised skin carry a higher risk of subsequent infection and thus, implant infection (Nunamaker, 2000).

For internal fixation to be successful, anatomic reconstruction of the bone and joint surfaces that allows for sharing of load between the reconstructed bone and the implants is essential (Nunamaker, 2000). This can be accomplished by screws alone or screws in conjunction with a plate. Interfragmentary compression is required for maintaining contact between fragments, which helps to protect the relatively weak implants. Small gaps in the reduction may increase the risk of implant failure through cyclic loading that occurs during normal weight bearing. Orthopedic implants alone are not capable of withstanding the full force of weight bearing in equine patients. Interfragmentary compression creates forces perpendicular

to the fracture planes and prevents movement of individual pieces of bone. These forces in turn create large frictional forces that prevent sliding of the fracture fragments over one another (Nunamaker, 2000). Interfragmentary compression occurs whenever two fracture surfaces are pressed together tightly. Plates can be used to provide axial compression across transverse fracture planes either by the use of tension devices or by the insertion of screws in load in a dynamic compression plate. This axial compression is often combined with additional interfragmentary compression provided by the placement of lag screws in fractures that have transverse and oblique components (Nunamaker, 2000). Plates are strongest in tension and compression and weakest in bending. The plate-screw-bone construct is also weak in torsion due to the screws that secure the plate onto the bone. In order to minimize bending forces and have tensile forces applied across the plate, they should be placed on the tension side of the bone. When bone is subjected to a bending force, the bending force can be converted to a tensile force in the plate as long as the cortex opposite of the plate is intact (Nunamaker, 2000). A dynamic compression plate applied to the tension side of the bone is called tension band plating. Under dynamic load it converts the tensile forces into axial compressive forces. The tension band acts as additional compression placed on the bone to offset eccentric loading and thus help reduce or eliminate the tensile bending stress. If plates are placed on the compressive side, the resulting forces for the plate would tend to further distract the bone fragments on the side normally experiencing tensile loads (Rybicki, 1977). This would also increase the risk of the plate bending and subsequent implant failure. Without an intact cortex on the compression side, the tension band principle cannot work because of lack of buttress (Nunamaker, 2000).

1.4 Hydroxyapatite

Pure hydroxyapatite powder is commercially available with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Hydroxyapatite is crystallographically characterized as an apatite after sintering (Klein, 1983). However, the biomechanical properties of sintered hydroxyapatite are poor. A compressive strength of up to 600-700 MPa may be achieved and a tensile strength of up to 200-250 MPa, however, the resistance against fatigue failure is very low (de Groot, 1987). For this reason, hydroxyapatite is considered useless as a self-supporting material for making a load bearing prosthesis. Hydroxyapatite implants can only be used if either no force, or only compressive forces will be applied to the implant. With this in mind, the trend has been the use of thin coating of hydroxyapatite over metallic implants to combine the bioactive properties of hydroxyapatite with the mechanical benefits of the metallic implant.

The technique most often used to apply hydroxyapatite to metallic implants is known as plasma spraying. The plasma spray technique allows for intimate connection between the metallic implant and the hydroxyapatite. Plasma spraying was first described by de Groot in the late 1980's. A direct current (DC) electric arc is struck between two electrodes, while a stream of mixed gases passes through this arc. The result is an ionized gas of extremely high (up to 30,000°C), with a high speed, approaching the speed of sound (300m/s) due to the large expansion resulting from this temperature increase (de Groot, 1987). This ionized gas is known as the plasma flame. Hydroxyapatite powder is suspended in a carrier gas stream which is then fed into the plasma flame. The hydroxyapatite particles melt in the flame and solidify upon impact with the metal implant, building up layers of particles. A chemical bond is formed between the hydroxyapatite and the implant. Grid or bead blasting of the metal surface prior to plasma spraying can be used to enhance bonding of the hydroxyapatite (Hermann, 1988). Appropriate temperature of the plasma flame is crucial to hydroxyapatite application. If the

temperature is too high the hydroxyapatite particles may vaporize and if the temperature is too low some particles will fail to sufficiently melt, leading to unbound particles in the lamellar structure. Other variables can also affect the quality of the hydroxyapatite coating on implants; these include particle size of the hydroxyapatite powder, plasma flame velocity, distance between the spray gun and metallic implant, and the pressure of the carrier gas (Hermann, 1988).

Hydroxyapatite has been a focus in human implantology since the late 1980's. It has been used in orthopedic as well as dental implants. Cementless total hip replacements are possible with good long term outcomes as a result of the development of hydroxyapatite coated implants (Soballe, 1993). Hydroxyapatite coating of transfixation pins increases stability and thereby reduces the risk for pin tract infection and mechanical failure of external fixator fracture fixation in human patients (Moroni, 1998). In the face of instability, implants develop a fibrous membrane surrounding them, whereas with stable implants, there is a variable amount of bone ingrowth (osteointegration). It has been shown in a canine model that hydroxyapatite coating resulted in osteointegration of implants even in the face of instability when examined at 16 weeks (Soballe, 1999).

Comparing insertion and extraction torque of a cortical screw or transfixation pin in vivo studies, using a sheep model, can provide a biomechanical measure of implant stability and osteointegration into the hydroxyapatite coating (Moroni, 2002 and 2008). A decrease in extraction torque compared to the insertion torque suggests that bone resorption and fibrous tissue deposition at the bone screw interface (BSI) has taken place during the given time period. An increase in extraction torque compared to the insertion torque, however, suggests that osteointegration has taken place during the given time period. Results from a recent in vivo study indicate that this process appears to occur with the placement of HA-coated screws in dorsal

equine MC3 bones under loaded conditions as well, with extraction torques being significantly increased when compared to insertion torques at both 8 and 12 weeks post-implantation (Gudehus, 2009). In this same study, the electropolished AO cortical bone screws had a significant decrease in extraction torque when compared to insertion torques at 8 and 12 weeks post-implantation.

Thickness of the hydroxyapatite coating is also important and must be a compromise between mechanical properties and resorption of the hydroxyapatite. One aspect is that the thinner the ceramic coating, the better its mechanical properties (de Groot, 1987). However, it has also been shown that within the first few months of implantation 15-20 μm of a hydroxyapatite surface may dissolve as calculated by changes in average pore diameter (de Groot, 1987). Continuous micromotion seems to increase the amount of resorption. In a canine model unstable implants had significantly more hydroxyapatite resorption than their stable counterparts (Soballe, 1999). A coating thickness of 50 μm is generally accepted as a compromise of lowering the risk of hydroxyapatite coating fracture and maintaining a porous structure on the implant's surface (de Groot, 1987).

1.4.1 The 5.5 mm AO Cortical Bone Screw

The 5.5 mm cortical screw (Synthes Vet, Paoli, PA) is made of 316 L stainless steel and is fully threaded. It is not self-tapping so a tap is required to cut its threads into the bone before insertion. The drill bit for the thread hole measures 4.0 mm in diameter. The thread diameter of the screw is 5.5 mm and the core has a diameter of 3.9 mm. They are commercially available in lengths from 24 to 100 mm (Synthes Vet Catalog, 2008). Electropolishing of stainless steel is considered standard, which gives it a smooth surface with a roughness average of 0.13 μm . This

surface is void of microdiscontinuities and as a result supports fibro-osseous integration upon implantation (Hayes, 2010).

1.4.2 The Hydroxyapatite Coated Screw

Other than the surface coating of hydroxyapatite that was applied, the 5.5 mm hydroxyapatite coated screw is identical to the 5.5 mm AO cortical screw. The surface of the threaded portion of the 5.5 mm cortical screw was roughened with aluminum oxide, ultrasonically cleaned, and plasma spray coated with HA with a mean thickness of 50.0 ± 10.0 μm (Orchid Bio-Coat Inc, Southfield, MI). The thread surface of the screw was roughened to improve bonding of the HA to the threads. Test screws were plasma sprayed coated with HA and measured at the shaft and thread surface to ensure the coating thickness was 50.0 ± 10.0 μm

CHAPTER 2. EVALUATION OF PLASMA SPRAYED HYDROXYAPATITE COATED AO CORTICAL SCREWS IN EQUINE THIRD METACARPAL BONE

2.1 Introduction

A goal of internal fixation is to maintain fracture stability in order to encourage bone union while maintaining a functional limb during healing (Beinlich and Bramlage, 2002). In a recent retrospective study of the surgical management of complete diaphyseal third metacarpal (MC3) and third metatarsal (MT3) bone fractures in the horse, the most common cause of internal fixation implant failure was screw loosening, bending or breaking (Bischofberger et al, 2009). This suggests that the weak link of long bone internal fixation in horses is the bone-screw interface (BSI). Bone resorption and implant loosening is often the result of cyclic mechanical loading during limb use (Zacharias et al, 2007). Other factors that affect bone resorption at the BSI include mechanical and thermal damage during screw insertion.

Hydroxyapatite (HA), the principle inorganic component of bone matrix, provides a rigid structural scaffold for the organization of the organic components of bone (Zacharias et al, 2007). Hydroxyapatite coating of external fixation pins has been used to prevent pin loosening in humans by the promotion of osteointegration, the process of bone ingrowth into the HA coating. (Aro et al, 1995 and Magyar et al, 1997). Results from a previous study evaluating HA coated cortical screws indicate that this process appears to occur in equine MC3 as well, with extraction torques being significantly increased when compared to insertion torques at both the 8 and 12 weeks post implantation (Gudehus et al, 2009).

The objective of this in vivo study was to compare the extraction torque of hydroxyapatite coated cortical screws to standard, polished screws placed in conjunction with a 4-hole dynamic compression plate (DCP) inserted unicortically in the dorsal diaphysis of the equine MC3. We

hypothesized that mean extraction torques of the plasma sprayed HA coated 5.5 mm cortical screws inserted in equine MC3 bones would be significantly greater than the insertion torques after 12 weeks of healing. Additionally, we hypothesized that the mean extraction torque of the standard AO screws would be significantly lower than the insertion torque after 12 weeks of healing.

2.2 Materials and Methods

Six adult Thoroughbred horses between 2 – 7 years of age, free of orthopedic disease, were included in the study group. The animals were housed in single, 3 m x 3 m box stalls throughout the initial study period and horses were allowed access to free choice water and grass hay and supplemented with 8 pounds of a complete pelleted diet (Horse Chow™, Purina Mills, St. Louis, MO) per day, divided in 2 feedings. In the initial postoperative period (14 days), the horses were stall confined. Following suture removal, the animals were group housed in a 100m x 100m pasture until the time of implant removal. The animals were assessed daily for general comfort and orthopedic soundness by the primary investigator throughout the study period.

Food but not water was withheld 12 hours prior to surgery. An intravenous jugular catheter was placed using aseptic technique. Perioperative medications consisting of potassium-penicillin (22,000 IU/kg IV), gentamicin (6.6 mg/kg IV), and flunixin-meglumine (1.1 mg/kg IV) were administered 30 minutes prior to induction of general anesthesia. Xylazine (0.5 mg/kg IV) and butorphanol tartrate (0.02 mg/kg IV) were administered for preanesthetic sedation.

Anesthesia was induced with diazepam (0.5 mg/kg IV) and ketamine hydrochloride (2.2 mg/kg IV), and maintained on total intravenous anesthesia with triple drip (1L guaifenesin, 500mg xylazine, and 1g ketamine) at 2.2ml/kg/hr adjusted as needed based on clinical monitoring.

Horses were placed in dorsal recumbency and the surgical site was routinely clipped, prepared and draped.

From a group of forty-eight 24mm long 5.5mm AO cortical screws (Synthes, West Chester, PA) twenty-four were randomly assigned to have a plasma sprayed HA coating applied to the threads with a mean thickness of $50.0 \mu\text{m} \pm 10.0$ (Orchid Bio-Coat Inc, Southfield, MI) (Figure 1).



Figure 2.1. Plasma sprayed HA coated 5.5 mm AO cortical screw (left), uncoated 5.5mm AO cortical screw (right).

The remaining twenty-four 24mm long 5.5 mm AO cortical screws served as the control. For each horse, four HA coated screws were inserted on the dorsal cortex of a randomly chosen MC3

bone, with 4 uncoated screws inserted in the dorsal cortex of the contralateral MC3 bone. All screws were placed through a 4.5mm broad DCP.

For each limb, the mid-diaphyseal area was determined as the midpoint between the carpometacarpal and metacarpophalangeal joint and a single incision was made in the dorsal mid diaphysis, using a 4-hole broad dynamic compression plate (DCP) as a template. Screws were placed using standard ASIF technique. Specifically, holes were drilled perpendicular to and through the dorsal cortex of the MC3 bone using a 4.0 mm drill bit (Synthes, Paoli, PA) using a drill guide in the neutral position. The depth of each hole was measured using a depth gauge and the thickness of the dorsal cortex recorded. Each hole was tapped using a 5.5 mm tap. A new 4.0 mm drill bit and 5.5 mm tap was used for each limb. Drilling, tapping and screw placement were performed under constant irrigation using 0.9% NaCl solution. Screws were inserted by hand using a screw driver with the final revolution using a digital torque wrench (DTW 265i, Checkline, Cedarhurst, NY) fitted with the shaft of an AO/ASIF screwdriver that was welded to a 3/8 inch square drive socket adapter. All screws in the plate were tightened to a final torque of 5.4 N-m. Incisions were closed in two layers using 2-0 polydioxanone (PDS) (Ethicon, Cornelia, GA) in a simple continuous pattern for the subcutaneous tissue and 2-0 nylon (Ethilon) (Ethicon, Cornelia, GA) in a simple continuous pattern for the skin.

Both limbs were bandaged and the horses recovered from general anesthesia under assistance using head and tail ropes. Bandages were changed every other day until suture removal 14 days postoperatively.

For implant removal at 12 weeks, horses were anesthetized in an identical manner as used for implant placement, placed in dorsal recumbency, and the surgical area clipped, prepped, and draped. A single incision was made directly over the plate extending 0.5cm beyond either end of

the plate. Screws were removed and extraction torques recorded using the same digital torque wrench used at the time of screw placement. The plates were removed using a combination of periosteal elevator and osteotome as needed. Incisions were closed as previously described and the limbs bandaged. Bandages were maintained until suture removal 14 days post operatively.

Statistical analysis

Mean \pm standard deviation (SD) was calculated for the extraction torques for each screw type. Paired samples were evaluated using *t*-tests for paired sample means within each testing group (Smith’s Statistical Package, Claremont, CA). Statistical significance was set at $p < 0.05$.

2.3 Results

Results from 5 horses were included for statistical analysis. At 12 weeks, the mean extraction torque of the HA coated screws was significantly ($p < 0.0002$) greater than the standard insertion torque of 5.4 N-m (Table 1). The mean extraction torque of the uncoated screws was significantly ($p < 0.0001$) less than the standard insertion torque of 5.4 N-m (Table 1). The mean extraction torque for the HA coated screws were significantly ($p < 0.0001$) greater than that of the uncoated screws (Table 1).

Table 2.1. Mean \pm SD Extraction Torques and Dorsal Cortex Thickness (5 horses).

Screw Type	Cortical Bone Thickness (mm)	Extraction Torque (N-m)
Uncoated AO	20.0 \pm 1.15 ¹	1.56 \pm 0.50 ^{2,3}
HA Coated AO	20.3 \pm 0.72 ¹	7.46 \pm 2.03 ^{2,4}

¹ There is no significant ($p = 0.33$) difference between the mean cortical bone thickness for the HA coated and uncoated screws.

² The mean extraction torque for the HA coated screws is significantly ($p < 2.36 \times 10^{-11}$) greater than that for the uncoated screws.

³ The mean extraction torque for the uncoated AO screws was significantly ($p < 1.42 \times 10^{-18}$) less than 5.4 N-m

⁴ The mean extraction torque for the HA coated screws was significantly ($p < 0.00002$) greater than 5.4 N-m

No signs of lameness were observed in the immediate postoperative period following implant placement or extraction. Mild incisional seroma formation occurred in two horses which caused no discomfort and resolved without further treatment. At the end of the study period, all incisions had healed normally; however, all horses had a noticeable change in contour of the dorsal cannon bone consistent with periosteal reaction and soft tissue scar formation at the surgery site. These areas were not painful to palpation and no lameness was noted.

One horse was noted to have partial dehiscence and incisional drainage post operatively. Radiographs showed no bony abnormalities; however, implant infection was suspected. Aerobic culture of all screw holes was performed at the time of implant removal. The proximal three screw holes cultured positive for a gram negative rod. This horse was excluded from all statistical analysis.

2.4 Discussion and Conclusions

The success rate of internal fixation of adult equine long bone fractures still remains poor. In a recent retrospective study, of the adults horses having complete diaphyseal third metacarpal (MC3) and third metatarsal (MT3) bone fractures stabilized by internal fixation supplemented with either a transfixation pin cast or cast, survival was achieved in only 38% of the cases and only 25% were fit for their intended activity (Bischofberger et al, 2009). Despite

this, other than the 5.5 mm cortical screw and a prototype tapered shaft 5.5 mm cortical screw (Sod et al, 2006) there have been no recent advances in screw design for the equine patient. This provided the motivation, in part, to focus on improving the stability of the cortical screw under cyclic mechanical loading. The decision to consider plasma sprayed HA coated cortical screws was based on the use of a transfixation pin cast or a taper-sleeve transcortical pin external skeletal fixation device for the stabilization of distal radial, third metacarpal (MC3), third metatarsal (MT3), and phalangeal fractures (Lescun et al, 2007; Elce et al, 2006 and Nunamaker and Nash 2008). For these external fixation devices, pin loosening poses a significant problem for the equine patient (Zacharias et al 2007), and the use of HA coated implants to improve stability at the bone-implant interface has been the focus of several studies (Zacharias et al, 2007; Magyar et al, 1997, and Soballe et al, 1993) .

Cortical bone screws are widely used for internal fixation of fractures, either alone (applied in lag fashion) or to secure plates. Since a 316L stainless steel cortical bone screw and equine cortical bone have a different modulus of elasticity, the stresses and strains are concentrated at the BSI (Ling 1986) . Thus, the weak link of internal fixation in horses is the BSI (Bischofberger et al, 2009). Reducing the amount of motion of the screw during the immediate postoperative period can decrease screw failure and lead to bony incorporation of HA coated implants (Soballe et al, 1993). Histological studies have shown that immediately after its insertion, a limited area of contact between screw threads and bone exists (Uthoff, 1973). Only at the level of the horizontal thread surface of an AO cortical screw, which is oriented towards the head of the screw, do the threads firmly oppose the bone (Figure 2.2).

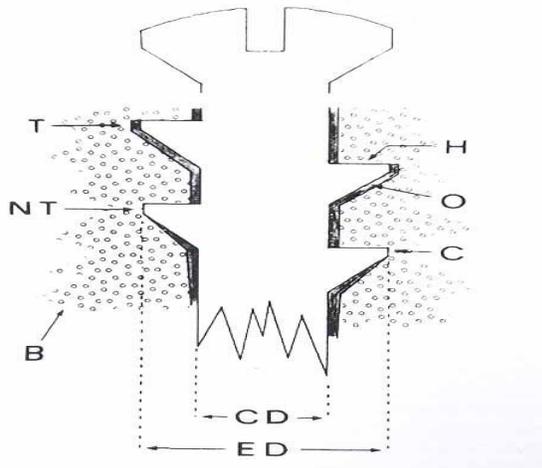


Figure 2.2. Diagram showing that the contact between screw and bone (B) - is limited to a portion of the horizontal thread surface (H). The oblique (O) undersurface of the screw thread is separated by a space shown as dark strip. When a tap has been used, the thread crest does not touch the bone (T), whereas, when a self-tapping screw is inserted, the thread crest does touch the bone (Redrawn from: Uthoff HK: Mechanical factors influencing the holding power of screws in compact bone. *J Bone Joint Surg* 55B, 633-639, 1973).

The contact between the horizontal thread surface and bone is partly caused by tightening the screw (placed in load or neutral) to the plate or the trans-cortex (Uthoff, 1973). In tightening the screw, the horizontal surface compresses the bone while the oblique under-surface of the thread is lifted away from the bone. For traditional AO screw insertion, the thread hole (3.2 mm for the 4.5 mm and 4.0 for the 5.5 mm cortical screws) is larger than the inner core diameter of the screws (3.1 mm for the 4.5 mm and 3.9 mm for the 5.5 mm cortical screws), which results in decreased bone thread depth and less contact. The diameter of the tap is slightly larger than the thread diameter of the screw. While this allows for less resistance during screw insertion, there is less contact between the screw thread and bone allowing for more micromovement between the two. These spaces between the screw threads and bone can be up to 0.150 mm thick. The limited area of contact and the presence of spaces between screw and bone can permit micromovement of the screw in its bed resulting in fibrous tissue incorporation rather than

osteointegration (Uthoff, 1973). To improve the fit between the screw thread and bone the use of cortical bone screws having plasma spray hydroxyapatite (HA) coated screw threads was considered.

Unicortical screw placement was chosen in this study in order to allow utilization of a single length 5.5 mm cortical screw. In a typical clinical setting cortical screws are inserted bicortically and inserting the screws unicortically created a less stable model than if the screws been inserted bicortically. Additionally, these screws were loaded through a plate to further stress the BSI. When loaded through a plate screws undergo greater cyclic loading and thus increased movement at the BSI. Our results showed that there was still a significant increase in extraction torque for the HA coated screws despite this additional movement at the BSI.

Increasing the net diameter of a screw by coating its surface has proven beneficial by reducing immediate postoperative motion; it increases the primary stability after insertion (Sod et al, 2007). A 4.5 mm broad LC-DCP fixation of an osteotomized equine MC3 bone secured with Teflon tape wrapped threads of cortical screws resulted in a 2.9 fold increase in the number of cycles to failure compared to the fixation secured with standard cortical screws (Sod et al, 2007).

Comparing insertion and extraction torque of a cortical screw (Moroni et al, 2002) or transfixation pin (Moroni et al, 1998) in vivo can provide a biomechanical measure of implant stability and osteointegration. A decrease in extraction torque compared to the insertion torque suggests that bone resorption and fibrous tissue deposition at the BSI has taken place during the given time period (Moroni et al, 2002). While an increase in extraction torque compared to the insertion torque suggests that osteointegration has taken place during the given time period (Moroni et al, 2002). In the case of the HA coated screws there was a significant increase in the mean extraction torques compared to the insertion torques at 12 weeks. This suggests that

osteointegration was occurring by 12 weeks. At the time of implant removal there was an audible pop as the screws were loosened which was immediately followed by a rapid decrease in torque. We feel that this correlates with the break-down of the osseous attachment that had formed between the cortical bone and the implant. Scanning electron microscopy performed at the time of implant removal provided visual evidence of bone ingrowth into the HA coating further indicating that osteointegration had occurred at this 12 week time point (Figure 2.3).

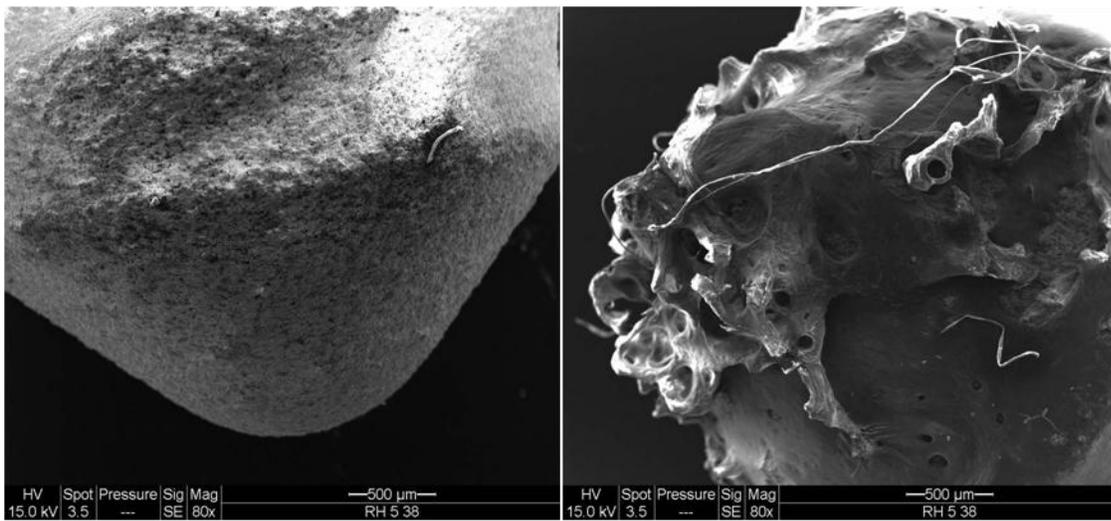


Figure 2.3. SEM of plasma sprayed HA coated 5.5mm cortical screw prior to implantation (left) and following removal at 12 weeks (right)

The results of this study suggest that the use of plasma sprayed HA coated screws may provide a means of improving the stability of internal fixation of an equine long bone fractures during both the early healing period (before osteointegration occurs) and during the later healing period (after osteointegration occurs).

CHAPTER 3. FINAL DISCUSSION AND CONCLUSIONS

3.1 Summary

Although this study was not a fracture model, we were able to evaluate the mechanical strength of the bone-screw attachment and found that HA coated screws have a significantly greater extraction torque when compared to the AO cortical screws. The potential increase in stability resulting from using HA coated screws would aid in equine fracture repair. However, a limitation of using HA coated screws could exist by the possible continued integration with the bone as the fracture heals. This could result in an integration that is too solid following fracture healing, so that screw removal would become difficult or impossible. Further investigation is required in an in vivo model to determine the extent of osteointegration associated with the HA coated screw in equine bone. Ideally this model would employ the use of osteotomies to more accurately reproduced healing in a fracture environment. Histology and histomorphometry would be beneficial in future studies to determine the type of tissue that comes in contact with the screw and measure the degree of bone-implant contact.

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