2010

Applicability and integration of plasma sprayed hydroxyapatite coated AO cortical bone screws in equine bone

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APPLICABILITY AND INTEGRATION OF PLASMA SPRAYED HYDROXYAPATITE COATED AO CORTICAL BONE SCREWS IN EQUINE 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 Science

in

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

by

Timm Hilmar Gudehus
Ludwig-Maximilians-Universität-München, Munich, Germany, 2003
May, 2010

DEDICATION

To my parents, Sabine Gudehus and Dr.-Ing. Helmut Gudehus for their continuous love and support.

ACKNOWLEDGEMENTS

The author would like to thank his major professor, mentor and dear friend, Dr. Gary A. Sod for all his guidance throughout his graduate program. Dr. Sod was an incredible mentor and clinical teacher going beyond any requirements supporting the clinical and academic training in any possible way.

The author would also like to thank the other members of his committee, Drs. Charles T. McCauley and Colin F. Mitchell, for guidance and support during the research, as well as critical review of the manuscript and providing recommendations on its improvement.

The author would also like to take the time and thank the members of the surgery faculty at LSU-SVM, Drs. Daniel J. Burba, Colin F. Mitchell, Charles T. McCauley, Laura M. Riggs, Mustajab H. Mirza and again Dr. Gary A. Sod for their training support and mentoring throughout his residency program. The author would like to thank Drs. Wolf Erhardt and Norman W. Rantanen who gave me guidance and support as incredible mentors, becoming confident in building a professional personality in clinical and scientific efforts.

Finally, the author would like to thank his parents, Dr. Helmut and Sabine Gudehus as well as his sister Dr. Ulrike Ullrich, for their unbelievable support and to evoke and share his passion in horses, enabling him to achieve his goals, on a personal and professional base.

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ABSTRACT

Objectives – To compare insertion temperatures and torques of Hydroxyapatite (HA) coated and uncoated 5.5 mm AO cortical screws in equine third metatarsal bones (MTIII) *in vitro*, and to compare insertion and extraction torques of HA coated and uncoated screws after 4, 8, 12 and 16 weeks of healing in equine third metacarpal bones (MCIII) *in vivo*.

Results – No significant temperature differences were recorded in cadaveric bones for AO and HA coated screws. Insertion torques were significantly higher for HA coated implants compared to uncoated screws. *In vivo*, the AO screws lost 50% of their initial stability within 4 weeks of healing and failed to gain stability over the next 12 weeks. The HA screws maintained stability (at 4 weeks), and roughly doubled (at 8 weeks) and tripled (at 12 weeks) their insertional torques over time.

Conclusions – HA coated screws can safely be inserted in equine cortical bone. AO cortical stainless steel screws fail to maintain stability in equine cortical bone. The addition of HA coating to the screws enables active osseointegration over 3 months of healing, as indicated by significantly higher extractional torques after 8, 12 and 16 weeks respectively.

Clinical relevance – Screw failure can occur under acute load and cyclic fatigue indicating the need for improved stability in the equine patient. HA coating, leading to active osseointegration, is commonly used in human implants for this purpose. Varying results in equine models led to guarded acceptance of implant coatings amongst equine surgeons. Our results support the osseointegrative properties of HA coated screws in horses.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Osteology

Bone serves as the framework of the body and as lever and attachment of muscles, affords protection to certain viscera, stores minerals and contains marrow which is essential in blood cell formation and is therefore considered a hematopoietic organ. It protects inner organs and serves as insertion for tendons and muscles, thereby functioning as lever arm for locomotion. In cross section from superficial to deep, bone is composed of the periosteum, cortical bone, spongy bone, endosteum and enclosed within, the medullary cavity (Sisson and Grossman, 1975).

Chemically, bone consists of roughly 70% of inorganic minerals, mainly calcium and phosphorus with some impurities and traces. Twenty (20) % of bone are made up by the extracellular matrix, consisting of predominantly collagen I (95%), proteoglycans, glycosaminoglycans (5%) and bone specific proteins (osteopontin and osteonectin). On the cellular level, osteoblasts, osteocytes and osteoclasts are predominantly responsible for the metabolism of calcified substance. Osteoblasts derive from fibroblastic osteoprogenitor or mesenchymal cells and are responsible for the formation of osteoid, the organic matrix. As they become incorporated in the osteoid matrix, they are referred to as osteocytes. They differ in the amount of endoplasmatic reticulum and cytoplasmatic organelles, the osteocytes being less metabolically active. Osteoclasts derive from blood monocytes and are responsible for the majority of bone resorption. They are found in concavities termed Howship's lacunae (Markel and Lopez in Auer and Stick, 2005). The remaining 10 % of bone's wet weight is made of water.

As the mineral part provides the static stability, it is the fine collagen network and proteoglycans providing with the elastic properties and flexibility, leading to this highly anisotropic framework of locomotion. Anisotropic refers to the fact that bone has very different properties in its resilience to different forms of load (direction), being significantly more stable under compression than under tension (Markel and Lopez in Auer and Stick, 2005).

Ninety-five (95%) of the organic matrix is collagen (mainly collagen I). It is composed of three alpha chains forming a tightly folded helix. Each alpha chain consists of repeating tripeptides of glycine, praline, hydroxyproline and hydroxylysine. Multiple triple helices form the tropocollagen molecule that arrange in a collagen fibril by chemical bonds, mainly disulphide bridges (Markel and Lopez in Auer and Stick, 2005).

The relationship of proteinacious and mineralized substances deserves attention. The microscopic unit of lamellar bone is built by the "osteon". Every osteon comprises a central vascular channel, the Haversian canal surrounded by concentric lamellae. Five (5) to 20 special lamellae (each 3 to 7 µm thick) build one osteon. Each lamella consists of parallel collagen fibers arranged in bundles that are described in detail above. The repeated banding pattern of the three dimensional collagen fibrils leaves 0.1 µm long gaps. These gaps are filled with the basic mineral crystal hydroxyapatite (HA) (Hees and Sinowatz, 2000).

With help of electron microscopy, the architectural arrangement of HA was revealed: HA is about 0.1 µm long and approximately 0.05 µm wide, perfectly filling the gaps between collagen fibrils without mechanical irritation of their alignment. Mineralized substance is thereby interposed between the collagen bundles. The deposition of calcium and phosphorus crystals (HA) on the preformed matrix is a complex process that can best be understood in terms of the events that occur in solution systems. Crystal formation in general consists of two steps: The formation of the nucleus and growth and proliferation of the crystals around thereafter. Depending on temperature and (super-) saturation of the solution with the precipitating ions, this process is favored. The interacting ions must be correctly aligned for sufficient saturation and stable crystal formation to occur. Matrix components and enzymes that regulate mineralization

are provided by osteoblasts, saturation of the matrix is influenced by hormones and availability of the ions depending on the physiologic status of the individual (Boskey, 1994).

Osteoblasts and osteocytes are interposed between the concentric lamellae of an osteon and between adjacent osetons. As they are connected by numerous cytoplasmatic processes they form and intricate transport and communication system within the lamellar bone. It is this framework that regulates the mineral homeostasis between the cells and the systemic circulation under fine regulation of vitamin D, calcitonin and parathyroid hormone. Circulatory function is supplied by a fine interconnecting network of Volkman canals between adjacent Haversian canals and the abundant blood supply from the periosteum, endosteum and medullary cavity. In general, this highly differentiated architecture is a unique property of lamellar bone, the main component of cortical bone. Cancellous or spongy bone is less organized and more vascular and generally composed of woven bone (Koenig and Liebich, 2009, see Figure 1.1).

The composite of rigid (HA) and flexible (collagen, proteoglycans) components provides a material that is superior in properties to either one alone. Bone is more ductile than HA alone, allowing the absorption of greater energy before failure; however it is more rigid than collagen, which permits greater load bearing and stiffness. This composite and an oblique bundle orientation make bone gain the stability to withstand static and elastic properties (Hees and Sinowatz, 2000).

In summary, bone is a comparatively strong material. The strength of bone is about 1/10 that of steel. Its apatite structure is responsible for its excellent compressive strength. The strength of cancellous bone is generally less than 1/10 of that of cortical bone. Due to only mild differences in tensile and compressive strength for individual bones, the tensile and compressive

strength of human bone has been reported to be averaged at about 1 MPa (Perren in Mueller et al., 1991).

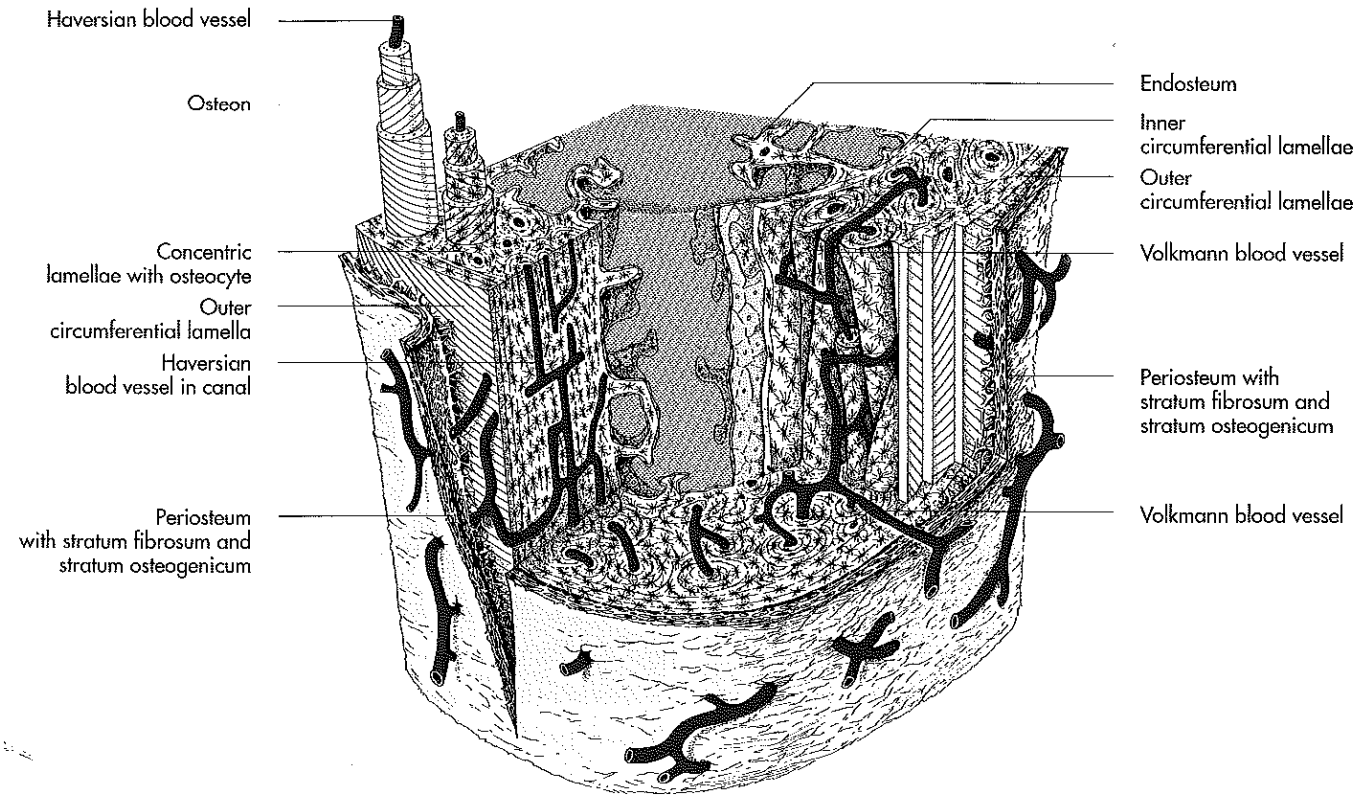

Figure 1.1. Microscopic picture of the histologic structure of bone (Picture taken from Koenig and Liebich, Veterinary Anatomy, Munich 2009)

1.1.1 Equine Cortical Bone

In its general architecture, the equine bone follows general osteology. Its cortical and trabecular densities have been determined to be 1035.25 mg hydroxyapatite/ml and 1048.55 mg hydroxyapatite/ml, respectively (Fuerst et al, 2008). The mean tensile strength of the equine third metacarpal bone has been reported to be 2137.9 to 2295.7 MPa (El Shorafa et al., 1979). In general, equine bone has a higher density in comparison to other species (Fuerst et al, 2008).

1.2 Fracture biology

Disruption of the architecture of bone can happen at the microstructural or macrostructural level. Its healing typically results in the return to the original form and function.

The involved processes are a temporary reversion to the embryonic state (Markel and Lopez in Auer and Stick, 2005). Mimicking embryonic development results in the difference of bone and soft tissue healing; bone healing resulting in a full regeneration o similar type tissue whereas soft tissues heal with a fibrous-type scar.

Due to trauma, there is separation of the osteonal structures with disruption of the integrity of blood vessels, causing hemorrhage at the fracture site. With the onset of intrinsic and extrinsic pathways of hemostasis and the added cellular components, quickly a cross linked clot forms limiting hemorrhage at the fracture site. This clot of finely intertwined fibrin fibrils is the earliest tissue bridging the fracture gap and serves as framework for incoming cells attracted by chemotaxis. Depending on the extent of the initial injury this clot can bridge periosteal, a unicortical defect, endosteal or across all cross sectional structures bicortically.

What follows is similar to healing in any tissue consisting of three distinct phases, inflammation, repair and remodeling. The inflammatory phase is the essential prerequisite for repair and fracture healing, usually lasting 2 to 3 weeks after the injury. As osteoclasts and macrophages remove mineralized and cellular/proteinacious components, they allow for ingrowth of mesenchymal cells. These, under the influence of fibroblastic growth factor (FGF), matrix metalloproteinases (MMPs), endothelial growth factors and many others, differentiate into endothelial cells, fibroblasts and osteoblasts, in an attempt to form blood vessels into the fracture gap along the scaffold of the fibrin clot. The fibroblastic, chondro- and osteoblastic activity leads to deposition of fibrous tissue, chondroid and osteoid, forming the soft callus. Through interposition of HA crystals, the early mineralized callus forms, leading to the formation of woven bone or hard callus. Depending on the extent of the initial injury this can be periosteal, endosteal, cortical or medullary. This process restores continuity at the fracture site using embryonic pathways and union occurs through endochondral and intramembranous ossification.

During and after this reparative phase, the remodeling phase (between 3 and 12 months after trauma) restores the osteonal structure, leading to formation of lamellar bone in cortical areas under the influence of tensile and compressive forces.

Depending on the amount of disruption or the quality of internal reduction and fixation, two distinct forms of healing have been discovered: Haversian remodeling or primary healing in areas of direct contact and secondary, indirect or gap healing in non contact areas. As indirect healing has to go through above mentioned complex pathways of repair and remodeling, it takes longer to restore the Haversian structure and previous tissue properties. Direct healing on the other hand allows for direct reconnection of disrupted osetonal structures with minimal remodeling allowing for earlier return to function. The ultimate goal from the surgeon's perspective is therefore to reconstruct the original cortical structure through adequate reduction, rigid fixation and sufficient blood supply (Hulse and Hyman in Slatter, 1993)

1.3 Bone Biology Surrounding Implants and Osseointegration

In the mid 19th century, Ollier and Barth simultaneously but independently studied the interaction between bone and different biomaterials (Wagner and Nawas, 2004). Branemark et al. (1969) investigated and achieved firm incorporation of tantal implants in rabbit femora and created the term "osseointegration". Schroeder et al. (1976) described the same observation under the term "functional ankylosis" (Wagner and Nawas, 2004). This interconnection between implant and surrounding bone achieved a high level of stability without interposition of connective tissue. It was recognized that throughout bony healing, newly formed Haversian canals are arranged according to the direction of axial and tensile forces. This alignment allowed for the direct transfer of axial forces to the surrounding bone. The implant, the bone-implant-interface and surrounding bone form one functional unit (Branemark et al. 1969). Stress on the

interface and surrounding bone, possibly leading to bony resorption, can thereby be avoided (Wong et al. 1995).

Therefore, osseointegration is defined as the direct structural and functional bond between organized living bone and the surface of a weight bearing implant (Wagner and Nawas, 2004). Others recognized that this tissue reaction interacting with the biomaterial is more complex; however it depends on several other factors such as chemical and physical attributes of the implanted material, structural properties of the implant surface as well as its macro structure, and the biodynamic influences under functional load (Knoefler and Graf, 1989).

Looking at the timeframe of fracture healing, the initial callus formation occurs within 1 week, leading to a bridging callus within 2-4 weeks and depending on the distance in between fracture fragments; this holds true for two opposing vital osseous structures. During implantation however, the existing gap between implant surface and surrounding bone has to be bridged entirely from the tissues' side; as the implant is not contributing to the healing process and aforementioned timeframes roughly double for any given distance (Knoefler and Graf, 1989). In terms of fracture biology, bone is facing an inert material and incorporation of the implant can only occur by gap healing. Indirect or gap-healing between bone and implant can be divided into four distinct processes (Von Rechenberg, 2004):

- The implant surrounding tissue is initially characterized through formation of a complex biofilm, predominantly consisting of blood components such as fibrin, a complex and heterogeneous group of proteases and thrombocytes (Wagner and Nawas, 2004). Cells in the post traumatic hematoma secrete inflammatory mediators such as prostaglandin E (PGE) and interleukins. These attract and initiate immigration of inflammatory cells (mainly neutrophils and macrophages) and mesenchymal cells, predominantly out of the

adjacent bone marrow. The thrombus becomes invaded by these cells and undergoes structural remodeling, leading to formation of granulation tissue. Interleukin 1 and 6, as well as PGE have an important influence on the activation of osteoclastic cells, initiating resorption of surgically traumatized bony tissue. Simultaneously, osteoblastic cells start secreting extracellular matrix. This matrix starts filling the initial surgically created gap around the implant (Von Rechenberg, 2004).

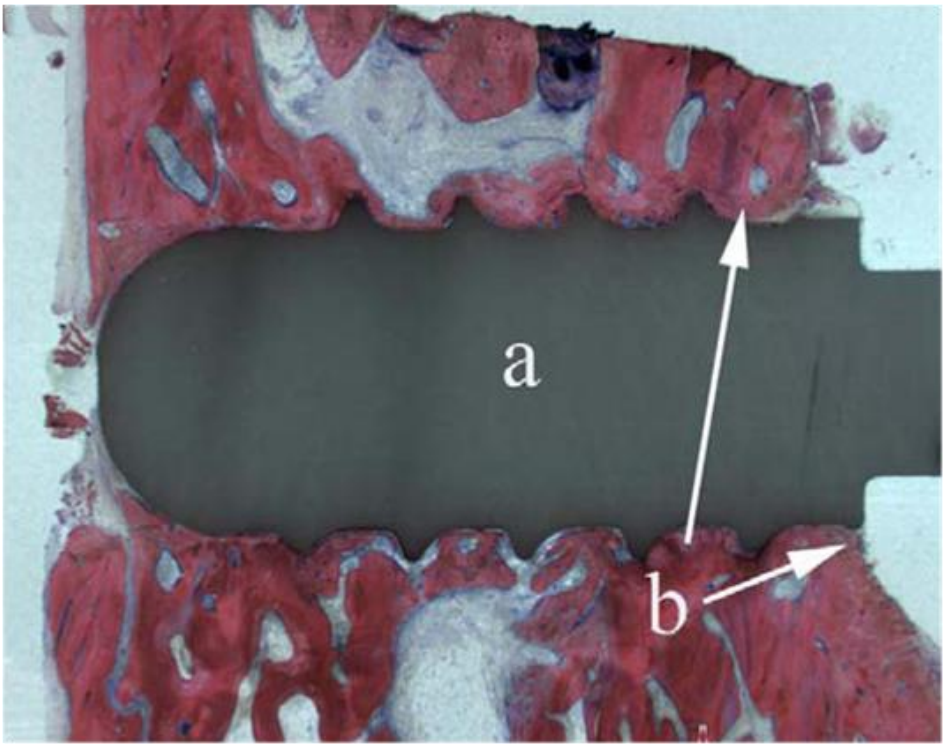

Figure 1.2. Microscopic picture of a SLA (sandblasted, acid-etched) Titanium tooth root implant in the maxilla of a miniature pig after 12 weeks of healing showing good, almost complete bony integration. The implant (A) in mineralized tissue (B) (HE stain, 10 X magnification) (Picture taken from Gudehus, 2006, Doctoral thesis, Germany).

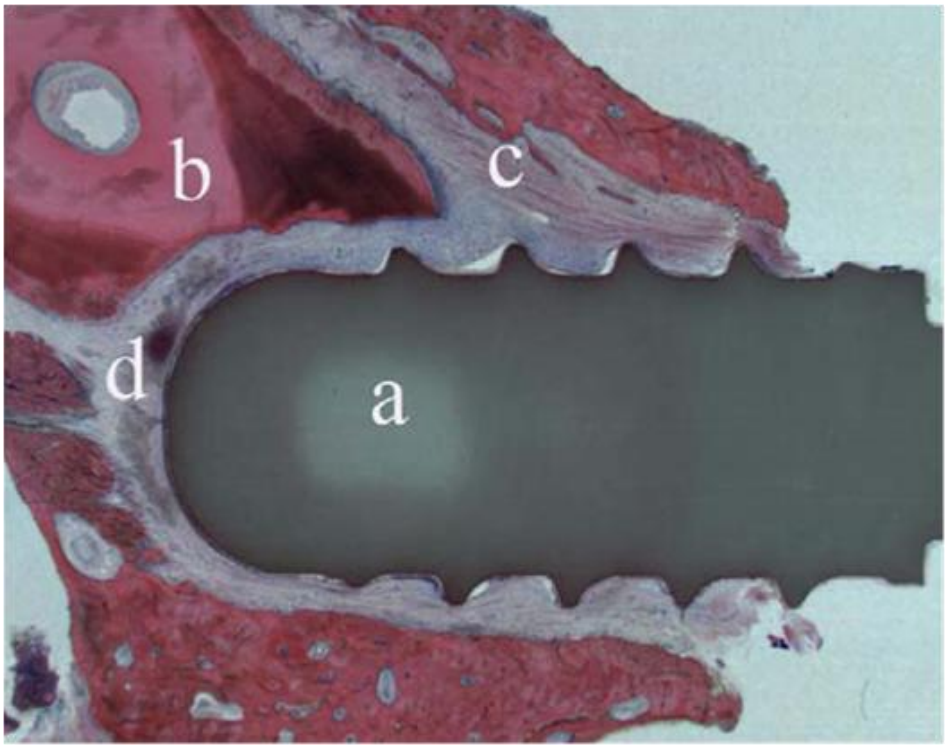

Figure 1.3. Microscopic picture of a SLA (sandblasted, acid-ached) Titanium tooth root implant in the maxilla of a miniature pig after 12 weeks of healing that failed to achieve osseointegration due to micromotion in the surrounding tissue. The implant (A) in fibrous connective tissue (C and D), with adjacent remaining tooth root (B) from formerly partially removed adjacent tooth (HE stain, 10 X magnification) (Picture taken from Gudehus, 2006, Doctoral thesis, Germany).

- By remodeling of the hematoma, an early soft callus forms, connecting the implant surface and surrounding bone. Fibrin has an important and complex role as it creates the initial posttraumatic seal between these two surfaces. However it also serves as a substantial and chemotactic scaffold for ingrowing, differentiating cells (Wagner and Nawas, 2004).
- Early mineralization and remodeling of a cartilaginous bridge leads to woven bone.
- As a final step, woven bone undergoes remodeling into lamellar bone.

During the integration of an implant with bone, there are two forms of osteogenesis: distance- and contact-osteogenesis. During distance osteogenesis, the osteoblasts migrate *towards* the implant surface, however do not incorporate into the implant surface, a gap between the biomaterial and this layer of osteoblastic cells remains. As they secrete osteoid and this matrix mineralizes, the implant becomes embedded in a calcified layer. The newly secreted bony matrix becomes interposed between the surrounding bone and the implant. This incorporation occurs from the outside in. During *contact-osteogenesis* however, undifferentiated cells migrate onto the implant surface and only differentiate into osteoblasts, while incorporated into the biomaterial's surface. Deposition of mineralizing osteoid originates from the implant's surface towards the surrounding bone, this is termed *from inside out* deposition (Steinemann et al. 1988). In reality however, assuming that forces on an implant throughout healing are not uniform, both processes are likely to occur simultaneously (Davies et al. 2003). Physiology of wound healing influences the weight bearing stability of the early post traumatic tissue (Steinemann et al. 1989): The initial tissue is characterized by inflammatory and resorptive processes that don't allow any transfer of load between the implant and surrounding bone. Only thereafter, an early load transfer onto the remodeling mineralized substance allows for minimal load. Around day 90 post implantation, force transmission becomes possible (Steinemann et al. 1989). The amount of implant-surrounding necrosis has to be considered; depending on thermal, mechanical and vascular factors, the initial surgical trauma always leads to a zone of dead bone, requiring resorption (Ling, 1986).

1.4 Internal fixation using plates and screws

Mueller, a Swiss orthopedic surgeon fostered the foundation of the "Arbeitsgruppe Osteosynthese (AO) in order to improve the clinical success of fracture repair incorporating fundamental research and clinical experience of this organization. At its outset in 1958, the AO

formulated four treatment principles (Mueller et al. 1991). They were: 1) *Anatomical reduction*, 2) *stable internal fixation*, fulfilling the local biomechanical demands, 3) *preservation of blood supply*, by atraumatic surgical technique and 4) *early active pain free mobilization* (Mueller et al. 1991).

Screw fixation is the heart of internal fixation using AO techniques, as they are designed to provide purchase in bone. They are designed to be loaded in tension, not bending or shear. Therefore they should be placed perpendicular to a fracture plane (using lag technique), which also distributes forces equally across the fracture plane. In conjunction with a plate, they should be placed perpendicular to the long axis of the bone if weight bearing loads are to be expected. The purchase of the screw generates compression of the screw head onto the plate. The result is frictional force at the screw's head's undersurface. This friction prevents the bone and plate from moving in relation to each other. It amounts to 37% of the axial force generated by the screw/plate combination; a greater number of screws will provide a greater bone/plate frictional force. Larger weight bearing loads will be tolerated before shifting between bone and plate occurs (Nunamaker, 2000).

The application and maintenance of compression between screw threads and bone are among the most important factor in attaining rigid internal fixation. If the compression decays rapidly, rigidity is lost and failure will occur. This loss can be divided into acute pull-out due to mechanical overload, secondary screw loosening under load over time or cyclic fatigue under the influence of healing (Schatzker et al. 1974).

Three major factors contribute to a persistently stable screw-shaped implant: 1) *Atraumatic insertion techniques*, as extensive necrosis surrounding the implant may render it impossible for the host to create osseointegration. 2) *The intrinsic nature of the implant*, as

different materials are able to induce varying host reactions (see chapter 1.4) and 3) *the mechanical environment between the implant and host*. Initially the mechanical circumstances between any implant and the host rely on the strength of the mechanical interlock. This depends on the strength of the surrounding tissue and the disposition of the implant's surface area. The balance between the strength of the interlock and the applied loads is essential (Ling, 1986). Uhthoff et al. (1976) showed that cellular differentiation relies on mechanical stability; in growing cells differentiated into osteoblasts, producing woven and later lamellar bone where fixation was stable and loads were adequate. Cellular differentiation into fibroblasts however occurs due to loss of stability (Schatzker et al., 1974). In conclusion, reduced micromovement and improved stability lead to osteogenesis and osseointegration, the most intimate and permanent contact between living bone and an implant (Ling, 1986).

1.5 Biomaterials and general surface characteristics

Biomaterials are either pure substances or combinations of natural or synthetic origin, except drug formulations, that are supposed to remain integrated as a treatment device to direct, supplement, and restore or replace tissue function for a predetermined timeframe (Wagner and Nawas, 2004).

An implant's biocompatibility describes the ability to elicit a biologically acceptable and predictable host response and can be subdivided in surface and structural compatibility. The surface compatibility includes the chemical, biologic and physical interaction between material and host tissue. The structural compatibility determines optimal adaptation to the implant's mechanical behavior in surrounding tissue. An implanted biomaterial therefore has to combine safety and functionality. These two terms summarize being inert, strong enough for the biomechanical properties required, easy to handle, noncorrosive, non allergenic, nontoxic, non

carcinogenic, easily sterilized, inexpensive and resistant to infection. The ideal biomaterial/ implant does not exist (Blackford et al. 2005).

Depending on the host' biologic response, materials can be classified as toxic, inert, resorptive or bioactive. Due to the nature of this thesis, only inert and bioactive will be further classified. Inert characterizes a non specific interaction leading to a non-adherent fibrous incorporation in both soft and hard tissues. No currently available implantable biomaterial is completely inert. All biomaterials elicit some form of host response; the degree however, varies greatly upon the implants' chemical properties. Bioactive materials on the other hand form an active interfascial bond with the surrounding tissue through time dependent modification of their surfaces (Blackford et al. 2005).

Regarding their reaction in bony tissue, bioactive materials can be further classified as osteoconductive, osteopromotive and osteoinductive. Osteoconductive is a material that serves as a scaffold, allowing bone to grow into it. Osteopromotive implants facilitate the bone's regeneration or promote it respectively. Osteoinductive materials induce new bone formation where mineralized tissue would not be synthesized physiologically (Wagner and Nawas, 2004).

Depending on the intended timeframe and achieved degree of incorporation, inert or bioactive materials are desirable for application in bony tissue. Stainless steel, titanium and composite materials consisting of ceramic coatings are currently used.

1.5.1 Stainless steel

One of the most extensively used orthopedic implant materials is ISO 5832-1 or ASTM F 138 (bar and wire)/ ASTM F 139 (sheet and strip). In the United States this is referred to as implant-quality 316 L stainless steel. It is an iron-based (62.5%) stainless composition out of wrought 17.6% chromium 14.5% nickel 2.8% molybdenum alloy, with minor other residual

alloys. Low amounts of non-metallic inclusions are ensured through special melting practices, which exclude secondary magnetic phases as much as elevated chromium and molybdenum levels. Different components in the alloy bring separate advantages. Chromium adds corrosion resistance, nickel provides microstructural stability and molybdenum improves resistance to pitting and crevice corrosion. The low carbon content (maximum 0.030% carbon) improves intergranular corrosion resistance (Disegi, 1994). Screws generally are supplied in cold-worked condition that permanently deforms the material at room temperature, reducing the cross sectional area through drawing or rolling which increases the implant's construct strength.

A passive layer (chromium oxide film) is present on the surface of stainless steel (SS), providing excellent corrosion resistance. Passivation in nitric acid (20 to 45% by volume) is commonly used to remove surface contaminants from the manufacturing process, as this restores maximal corrosion resistance. Immersing a finished steel implant in a chemical solution under application of a current removes a fine surface layer; this is referred to as electro polishing. A chemically passivated surface, improved corrosion resistance, lowered frictional coefficient and decreased surface roughness are the result (Blackford et al., 2005).

Whether these implant characteristics are desirable in the setting of equine long bone fixation will be discussed later in this work. Additionally, being completely non-magnetic makes stainless steel attractive for application as a long-term orthopedic implant (Blackford et al., 2005). Despite its relative inertness, the high Nickel content (15%) may provoke an allergic reaction, being responsible for up to 90% of the metal allergies observed in human patients (Hierholzer, 1992). Metal ion release may stimulate neutrophils. Release of lysosomal enzymes can lead to implant loosening over time, associated patient discomfort and aforementioned allergic reactions. The small implant particles or chemical components are phagocytized and

carried into regional lymph nodes. In the lymphoreticular tissue, the particles are released and can migrate or cause a foreign body reaction.

1.5.1.1 The 5.5 mm AO cortical screw

As all Synthes stainless steel screws, 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 (Nunamaker in Fackelmann, Auer and Nunamaker, 2000). 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 come in lengths from 24 to 100 mm (Synthes Vet Catalog, 2008). The thread hole diameter is 0.1 mm greater than the core diameter of the screw.

1.5.2 Hydroxyapatite

In 1965, Sandhaus presented and patented with alumina, also known as "Degussit Al 23", the first ceramic material as an implant for hip endoprosthesis (Rieger 2001). It has to be considered as the ancestor of all modern bioactive, ceramic based implants and surface textures. Hydroxyapatite (HA) was the next big breakthrough in the early 1970s, soon to be followed by tetragonal zirconia polycrystals (TZP) and aluminum oxide (Al_2O_3). Ceramic materials in general provide with excellent chemical purity and stability, corrosion resistance and density. They are highly inert or even bioactive (Rieger 2001).

Three types of ceramics can be differentiated: 1) *Oxide ceramics* such as Al_2O_3 and ZrO_2, have excellent tribological properties, low frictional coefficient and low wear rate. They find application for most hip systems as a ceramic ball with a conventional polyethylene socket counterpart. 2) *Glass ceramics* are silica based (SiO2), where soluble calcium phosphate ions are incorporated into the bioglass ceramic structure. A layer of HA is formed on the surface after implantation, providing a chemical bond between tissues and the implant surface. 3) *Calcium*

Phosphate Ceramics, such as tricalcium phosphate (TCP), octacalcium phosphate and HA resemble the mineral phase in bony tissue (reviewed by Soballe, 1993).

HA is crystallographically characterized as an apatite after sintering (Klein, Driessen and De Groot, 1983). Pure HA powder is commercially available with the chemical formula $Ca_{10}(PO_4)_6(OH)_{2.}$ Unfortunately the excellent biological properties of HA are combined with such inadequate mechanical stability (low coefficient of elasticity) that has been considered useless as a self-supporting material for constructing a load bearing prosthesis. Metallic implants remain the primary choice (Osborn, 1987). As a raw material it is used as granular bone filler for sites where loads are limited to pure compression (Osborn, 1985).

HA has been the focus of human implantology research for the past 3 decades, leading to a variety of applications as surface coating for titanium and its alloys as well as stainless steel for orthopedic, spinal and endodontic surgery. The idea is to combine the bioactive properties of HA with the mechanical benefits of a metallic core implant. Its application has made cementless hip endoprosthesis possible with excellent long-term results (Soballe 1993). Plasma-spray techniques have been developed, allowing for an intimate connection with the underlying metal (Osborn, 1987). The technique was first described by De Groot in the late 1980s (De Groot, 1987). Two electrodes form an electric arc and hydrogen or argon gas flows through the space between the electrodes, becomes ionized and forms a plasma flame. HA particles become injected at the speed of sound (300 m/s) into the high temperature plasma tail (15000 °C) and are driven against the substrate. The HA particles melt in the flame and solidify upon impact with the metal substrate, building up a layer of particles. Grid or bead blasting of the metal surface prior to plasma spraying can be used to enhance bonding (Hermann, 1988). The temperature of the plasma flame plays an important role, as too high a temperature may lead to vaporization of the particles and

too low a temperature will fail to sufficiently melt the particles which will lead to unbound particles in the lamellar structure of the metallic substrate. However, other variables determine the quality and homogeneity of the final implant such as particle size of the HA powder, plasma flame velocity, distance between the gun and the substrate as well as the pressure of the carrier gas (Hermann, 1988). An REM surface structure is shown in Figure 1.4.

Thickness of the coating has received considerable attention as it reflects a compromise between mechanical properties and dissolution (reviewed by Soballe, 1993). Mechanical properties increase as the thickness of the coating decreases. The amount of HA resorbed within the first year has been calculated between 15 µm and 50 µm. Bone bonding osteogenesis thereby results in loss of the apatite layer on the HA rather than resorption of the surface (Osborn, 1987). Continuous micromotion and load seem to increase the amount of degradation, leading to 77% resorption under load vs. 56% in immobilized controls in a canine *in vivo* model. The degraded HA however appears to be replaced at least in part by bone (Soballe, 1993). A coating thickness of 50-75 µm seems to be a generally accepted compromise, lowering the risk of HA fracture despite preservation of the porous structure on the implant surface (De Groot, 1987).

Good tribologic match between the modulus of elasticity of the metal core and apatite surface are essential. Strains between the HA coating and the metal substrate are minimized when the modulus of both components is as close as possible (Berndt, Haddad and Gross, 1989).

1.5.3 Biomechanical testing

When a force is applied to a implant-bone construct, where it is torsion, tension, or shear, displacement of the construct occurs. The displacement is measured as a change in length or area. A graph of the applied load versus the resulting displacement can be drawn, called a load-displacement curve as in Fig. 1.5. A simplified load displacement curve consists of three basic

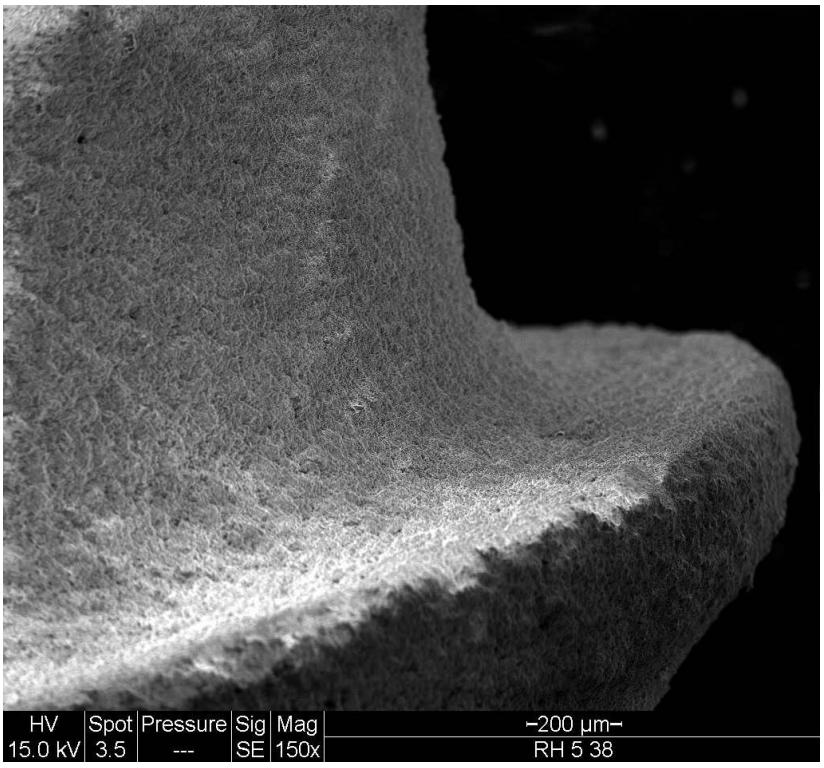

Figure 1.4. REM picture of the plasma spray coated implant used in this study before insertion into equine cortical bone

regions, the elastic, plastic and failure regions, each corresponding to ever increasing forces. The elastic region is the first region, which is characterized by a linearly relationship between force and displacement. The beginning of the elastic region has a preloading portion, which represents the actual engagement of the material testing machine. In this region, if the load is reduced to zero the displacement will return to zero and construct will revert back to its original configuration. The slope of the linear elastic region is called the stiffness of the construct with units of force per unit length (or area). As the load continues to increase, at point is reached where the there is no longer a linear but now a non-linear relationship between the load and the resulting displacement. This is the plastic region, where if the load is reduced to zero the displacement does not change, that is, the construct retains its most current shape. The load at which the elastic region transitions into the plastic region is called the yield load. If the load continues to increase the construct will continue to deform until a failure in the implant-bone

construct occurs. This is failure region is characterized by a abrupt decrease in load. The load at which the failure occurs is called the failure load.

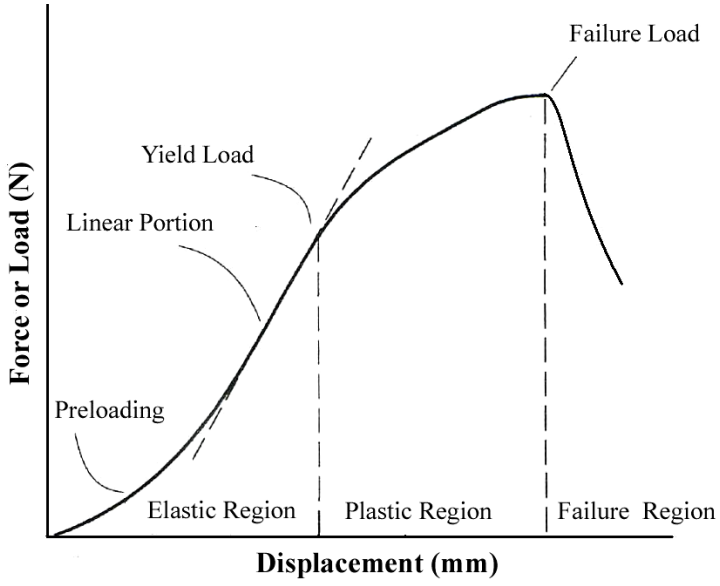

Figure 1.5. Typical load-displacement curve

Load deformation curves can be generated for different biomechanical evaluations using bending and torsion (Cornell, 1994), push-out testing (Schatzker, 1975, Rueger et al. 2010), pull-out and *torque-in* and *–out* measurements (Gahlert 2008).

During push- and pull out testing as well as *torque in* and *torque out* studies, the failure point represents the breaking of the bone- screw - (implant in general) interface. This is the point at which the surrounding bone fails. In case of pull– and push-out testing, the mineralized structure fails in-between the screw threads. This depends on the thread pattern and pitch of the implant as well as the net core size relationship of the implant and the core diameter of the drill bit (Abuhussein et al., 2009). In case of *torque-out* studies, the ultimate failure point is the failure of the fine layer of cells and mineralized structure apposed onto the implant's surface or interposed

between implant and surrounding bone (Gahlert et al., 2008; Moroni et al. 1996). The *torque-in* value thereby serves as a baseline representing the stability of an implant at the timepoint of insertion (Davies, 2003; Moroni et al. 1996, 1999, 2002). This torque reading has been correlated as a clinical predictive value for the degree of stability and achieved osseointegration (Trisi et al., 2009). It can also be used as comparison between the initial stability at the time of implant insertion, "primary stability", and the stability achieved at a time post-implantation, "secondary stability". Significant decrease or increase in *torque-out* compared to *torque-in* values can then be interpreted in light of successful repair and remodeling around an inserted biomaterial or surface structure. It thus allows for clinical evaluation of successful osseointegration or failure thereof (Moroni et al. 1996, 1999, 2002, 2008).

CHAPTER 2
AN IN VITRO ANALYSIS OF TEMPERATURES AND INSERTION TORQUES OF HYDROXYAPATITE COATED AND UNCOATED 5.5 MM AO CORTICAL SCREWS

2.1 Introduction

Providing initial stability and protection from fatigue caused by cyclic loading are the critical to the success of the fixation during fracture healing (Ling, 1986). The resistance of the bone-screw-construct to cyclic fatigue relies on the anchoring strength of the screw in the surrounding bone. Micromotion of the screw is responsible for toggling of the screw along its shaft, allowing it to back out over repeated cycles (Schatzker et al. 1975). This cycling behavior has been studied intensively allowing determination of the number of cycles to failure in comparison to known healing of fracture planes. No commercially available orthopedic implants provide sufficient cyclic stability to meet the healing time requirements in equine long bone fracture repair (Sod et al. 2008). Extensive research in human orthopedics has addressed the importance of macro- and micro texture of screws, providing better healing and osseointegration over time for loaded and unloaded implants (Thomas and Cook 1985; Boyan et al., 1999; Brett et al., 2004; Brunette, 1988).

There are few in vivo and in vitro studies in equine implantology investigating the contribution of the screws to the special stability of plate-screw-bone construct (Sod et al., 2005). Previous studies showed significant increase in the cyclic fatigue stability by wrapping screws in Teflon foil (Sod et al., 2007) or PMMA-luting (Sod et al., 2005). By providing the screws with a mechanically tighter fit, micromotion of the screws could be reduced, increasing cyclic fatigue behavior of tested implants in vivo (Sod et al., 2007). In a recent study, a 4.5 mm broad LC-DCP fixation of an osteotomized equine MC3 bone secured with plasma spray hydroxyapatite (HA) coated cortical screws was superior to uncoated cortical screws in resisting static overload forces and cyclic fatigue (Durham et al., 2010). These in vitro results suggest that HA coated screws improved the initial construct stability (Durham et al., 2010). HA, the principle inorganic

component of bone matrix, provides a rigid structural scaffold for the organization of the organic components of bone tissue (Soballe et al., 1993).

Critically high temperatures in the surrounding bones of HA plasma-spray coated pins resulted in the question whether these implants would be applicable in equine long bones (Zacharias et al., 2007). This resident and his co-researchers are unaware of any studies evaluating the insertional characteristics of HA coated cortical screws in equine cannon bones (MC/MT III).

The objective of this study was to determine if the mean maximum temperatures during insertion of HA coated 5.5 mm AO cortical screw in the cis and trans cortices would be significantly greater than the mean temperatures for an uncoated AO 5.5 mm cortical screw in equine third metatarsal bones (MT3). A second objective was to determine if the mean maximum temperatures obtained during insertion of the HA coated 5.5 mm cortical screws would exceed the physiologic values (53°C) (Abouzgia and James, 1997). Finally, we hypothesized that HA coated AO 5.5 mm cortical screws will achieve a higher end insertional torque along the threaded screw length.

2.2 Materials and Methods

Six pairs of equine MT3 bones were collected from Thoroughbreds (2 – 7 years of age), euthanized for reasons unrelated to orthopedic disease. The MT3 bones were wrapped in saline soaked towels and frozen at -20ºC within 4 hours of euthanasia. Before biomechanical testing, the specimens were thawed at room temperature (20-22°C) for 12 hours. In order to achieve near physiologic baseline temperatures, the paired MT3 (bone and enwrapping soft tissues) were placed in an incubator maintained at 37 °C for 4 hours prior to testing. The mid-diaphyseal soft tissues were stripped circumferentially including the periosteum. Each bone was mounted in a holding vice. Using a 3.2 mm drill bit, at the midpoint of the diaphysis, a hole was drilled into the

dorsal and plantar cortices to a depth of 5 mm, 7 mm from the premarked location of screw insertion, in order to accommodate an implantable temperature probes (model # KMQSS-032-6, Omega Engineering, Inc., Stamford, CT). The baseline temperature of the cis and trans cortex was recorded using the thermo couple attached to a hand-held thermo sensor (model # HH806AU, Omega Engineering, Inc., Stamford, CT). The thread hole for the 5.5 mm screw was drilled in dorsoplantar direction and tapped using AO/ASIF technique. All drilling and tapping was performed under constant irrigation using 0.9% NaCl solution (150 ml/ min) at room temperature. During the drilling process, the drill bit was backed out and cleaned 2 to 3 times. The screws were inserted by hand using a screw driver. Insertional torque was measured for every screw using a digital torque wrench (Checkline, Cedarhurst, NY) as the screw emerged a full turn from the trans cortex. The screw length was chosen so that the head was not engaged at the cis cortex to ensure that only frictional forces between the threaded part of the screw and surrounding bone were recorded. Immediately after emergence from the plantar cortex, the temperatures of the drill bit, tap and the screw tip were recorded using a surface contact thermocouple (part # SA1XL-K-120, Omega Engineering, Inc., Stamford, CT).

A paired t-test was used for comparison of all value mean ± standard deviation between AO cortical and HA coated screws for temperature and torque measurements. The p value was set at $p < 0.05$.

2.3 Results

For the cis cortex, there were no significant ($p = 0.68$, $p = 0.49$, $p = 0.84$, and $p = 0.28$) differences between the mean initial cortical temperatures, the mean maximum cortical temperatures during the drilling of the thread hole, tapping of the thread hole and screw insertion, respectively, between the MC3 bones assigned to the HA coated screws and the MC 3 bones

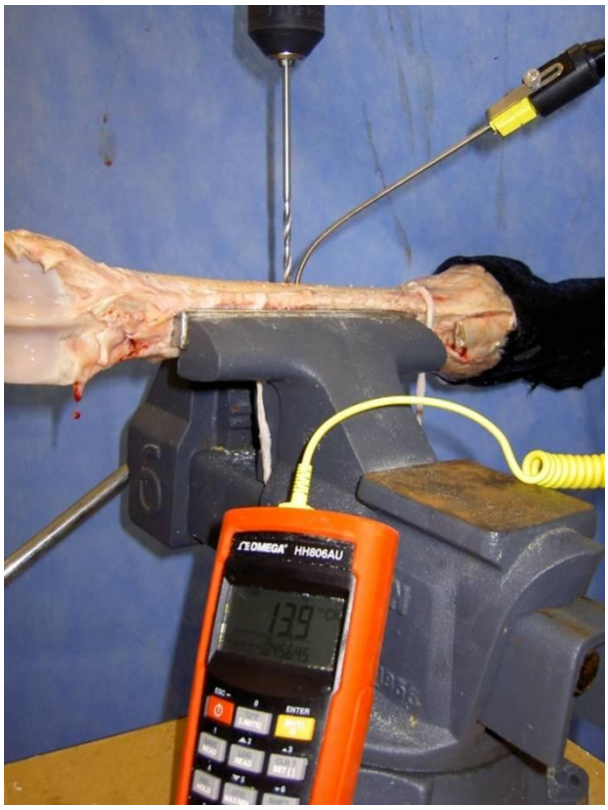

Figure 2.1. An equine third metatarsal bone in the testing device with thermocouples and the hand recorder in place

assigned uncoated screws (see Table 2.1). Similarly, for the trans cortex, there were no significant ($p = 0.24$, $p = 0.99$, $p = 0.99$, and $p = 0.23$) differences between the mean initial cortical temperatures, the mean maximum cortical temperatures during the drilling of the thread hole, tapping of the thread hole and screw insertion, respectively, between the MC 3 bones assigned to the HA coated screws and the MC 3 bones assigned uncoated screws (see Table 2.1).

There was no significant temperature difference recorded for the drill and tap tips protruding from the trans cortex (Table 2.2).The mean temperature of the protruding implant was significantly greater ($p < 0.04$) for the HA coated screws upon emergence from the trans cortex (Table 2.2).

The mean insertional torque was significantly ($p<0.00003$) greater for the HA coated screws than for the uncoated AO screws (Table 2.2).

2.4 Discussion

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). Reducing the amount of motion of the screw during the immediate postoperative period can decrease screw failure and lead to bony incorporation of the implant (Soballe et al., 1993). Histological studies have shown that immediately after its insertion, a limited area of contact between screw threads and bone exists (Uhthoff, 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 (Uhthoff, 1973). The contact between the horizontal thread surface and bone is partly caused by tightening the screw to the plate or the cis cortex, in the case of lag screw fixation. In tightening the screw, the horizontal surface compresses the bone while the oblique under-surface of the thread, which is oriented towards the tip of the screw, is lifted away from the bone (Uhthoff, 1973). 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 (3.0 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 tap often cuts threads in the bone whose diameter is larger than the outer thread diameter of the screw, which results in an even smaller area of contact between the screw thread and bone, with the crest of the screw threads losing contact with the bone. These spaces between the screw

Table 2.1 Mean ± SD values for comparison of mean initial temperature and mean maximum temperature post-drilling, post-taping, and post-screw insertion in cis- and trans-cortices of equine third metatarsal bones for HA coated and uncoated 5.5 mm cortical screws

	Cis Cortex		Tans Cortex	
Temperature (^{0}C)	**AO**	**HA**	**AO**	**HA**
Initial	30.6 ± 5.23 [1]	29.5 ± 2.36 [1]	26.8 ± 2.42[5]	28.5 ± 2.25[5]
Post Drilling	32.7 ± 4.57 [2]	31.1 ± 3.39 [2]	29.1 ± 3.02[6]	29.1 ± 2.67 [6]
Post Tapping	32.8 ± 4.13 [3]	32.3 ± 3.88 [3]	30.7 ± 3.36[7]	30.8 ± 2.95[7]
Post Screw Insertion	31.3 ± 4.02 [4]	33.6 ± 2.65 [4]	29.2 ± 3.24 [8]	31.3 ± 2.33 [8]

[1] There is no statistically significant difference (P = 0.68) for the mean initial temperatures at the cis cortex between MC 3 bones assigned to either screw type

[2] There is no statistically significant difference (P = 0.49) for the mean temperatures at the cis cortex during drilling between MC 3 bones assigned to either screw type

[3] There is no statistically significant difference (P = 0.84) for the mean temperatures at the cis cortex during tapping between MC 3 bones assigned to either screw type

[4] There is no statistically significant difference (P = 0.28) for the mean temperatures at the cis cortex during screw insertion between MC 3 bones assigned to either screw type

[5] There is no statistically significant difference (P = 0.24) for the mean initial temperatures at the cis cortex between MC 3 bones assigned to either screw type

[6] There is no statistically significant difference (P = 0.99) for the mean temperatures at the cis cortex during drilling between MC 3 bones assigned to either screw type

[7] There is no statistically significant difference (P = 0.99) for themean temperatures at the cis cortex during tapping between MC 3 bones assigned to either screw type

[8] There is no statistically significant difference (P = 0.23) for the mean temperatures at the cis cortex during screw insertion between MC 3 bones assigned to either screw type

Table 2.2 Mean ± SD values for comparison of mean maximum temperature of drill bit, tap, and screw for HA coated and uncoated 5.5 mm cortical screws

	AO	HA
Drill Bit Temperature (^{0}C)	33.7 ± 2.23[1]	32.6 ± 3.57[1]
Tap Temperature (^{0}C)	35.4 ± 2.34[2]	35.2 ± 5.09[2]
Screw Temperature (^{0}C)	31.9 ± 2.24[3]	39.5 ± 6.71[3]
Insertion Torque (N-m)	2.37 ± 0.94[4]	6.0 ± 0.58[4]

[1] There is no statistically significant difference (P = 0.53) for the mean temperatures of the drill bit for either screw type

[2] There is no statistically significant difference (P = 0.93) for the mean temperatures of the tap for either screw type

[3] The mean temperature of the HA coated screw is significantly ($p < 0.04$) than that of the uncoated screw after insertion

[4] The insertion torques of HA coated screws were significantly higher ($P<0.00003$) than for uncoated screws

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 (Uhthoff, 1973) which potentially can lead to fibrous integration of the implant under these unstable conditions (Clary and Roe, 1995).

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 and increases the chances for osseointegration (Soballe et al., 1993). 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 2.7 fold increase in the number of cycles to failure compared to the fixation secured with standard cortical screws (Sod et al., 2007). In a recent study, a 4.5 mm broad LC-DCP fixation of an osteotomized equine MC3 bone secured with plasma spray hydroxyapatite (HA) coated cortical screws was superior to uncoated cortical screws in resisting static overload forces and cyclic fatigue (Durham et al., 2010). These in vitro results suggest that HA coated screws improved the initial construct stability (Durham et al., 2010).

The almost 2.5-fold increase in insertional torque between the HA coated screws and the uncoated screws in this study could be due to the relative diameter increase of these implants (HA coating thickness 50 $\pm$ 10 μm) compared to the uncoated controls. This agrees with the findings of Zacharias et al who found a 1.4-fold increase in insertional torque between HA plasma spray coated and uncoated IMEX transfixation pins (IMEX Veterinary, Inc., Longview, TX) in equine metacarpal bones (Zacharias et al., 2007). An increased initial contact between bone and screw surface induces faster and more stable integration as well as better immediate and long term stability under load (Trisi et al., 2009).

The association between friction of implants and the bone and the thermal damage resulting in necrosis has long been recognized (Eriksson, 1983). Bone is a poor conductor of heat and the local temperature increase can be significant (Eriksson et al. 1984). The exact mechanisms that lead to cellular damage are not understood, but enzyme inactivation and protein denaturation have been shown to be involved. A recent review of the literature concludes that most authors agree that bone necrosis occurs beyond 50° C (Karmani, 2006). Differences are based on study designs, examined proteins and animal species. In the same review, individual proteins however seem to only denaturize at higher temperatures or after prolonged exposure time (Karmani, 2006). No studies have been conducted in equine models; the peak temperature tolerated in equine cortical bone thereby remains an empirical value.

Trying to elevate a formerly frozen specimen up to in vivo temperatures proves difficult despite incubation. The same problem is reflected in the baseline values by Zacharias et al. (2007). Using fresh cadavers and immediate testing after harvest might produce results closer to in vivo settings.

Differences between screw temperatures and elevations in cortical bone at a 7 mm distance (temperature probe) are most likely based on heat conductivity of bone (Morisset et al., 2000). It has been shown that measuring cortical temperatures at this distance as well as measuring the hardware temperatures upon immediate emergence from the trans cortex is a reliable method to assess temperature changes in the immediate surroundings of an inserted implant (Morisset et al., 2000). This protocol has been introduced in the past and is widely accepted (Morisset et al., 2000; Sod et al., 2005, Zacharias et al. 2007). Removal of the soft tissues allowed for equal cooling of both cortices. The increased exposure of cortical bone,

however, could have resulted in lower cis- and trans-cortex temperatures than one might expect in a surgical setting, particularly at the trans cortex.

Using a hand held drill introduces a certain degree of variability, however, the authors felt that the screw insertion technique should follow AO/ASIF techniques that would be used in a surgical setting. All procedures were performed by the same surgeon under identical settings. New drill bits and taps were used for every screw inserted in order to reduce variability in drilling and tapping.

Our results indicate that the mean temperatures at the cis and trans cortices during preparation of the screw holes as well as during screw insertion were not significantly different between the different screw types. None of the mean cortical temperature values exceeded 45º C (Table 2.1). Also the mean temperatures of the tips of the drill bits, taps, and screws were not significantly different between the screw types (Table 2.2).

Zacharias performed an in vitro comparison of the insertion temperatures of uncoated IMEX transfixation pins with transfixation pins having one of three different HA coatings under in vitro conditions. The mean temperatures of the pin during the insertion of plasma sprayed HA coated Imex pins were significantly greater than for the uncoated Imex pins (Zacharias et al, 2007). For the plasma sprayed HA coated pins, the increased insertional torque was accompanied by mean temperature of the pin greater the threshold for thermal necrosis, so the use of the HA coated pins was not recommended in the equine patient (Zacharias et al., 2007).

The significant difference between the mean temperature of the HA coated and uncoated pins following insertion is in disagreement with our results. To explain the differences we must look at the dimensions of the implants, drill bits and taps. The Imex pin has a shaft diameter of 6.3 mm and thread diameter of 8.0 mm. The drill bit (Imex Veterinary, Inc, Longview, TX) used

to drill the thread hole has a diameter of 6.2 mm and the tap (Imex Veterinary, Inc, Longview, TX) has a diameter of 8.0 mm. The resulting thread hole is 0.1 mm smaller in diameter than the shaft diameter of the pin which would result in a press fit during insertion. In contrast, the AO 5.5 mm cortical screw has a shaft diameter of 3.9 mm and thread diameter of 5.5 mm. The drill bit (Synthes, Inc, Paola, PA) used to drill the thread hole has a diameter of 4.0 mm and the tap (Synthes, Inc. Paola, PA) has a diameter of 5.55 mm. Thus a looser fit existed between the screw and bone during insertion than the pin. The HA coating adds 50 + 10 μm to the dimensions of both the screw and the pin. In the case of the HA coated screw, the coating resulted in partial filling of the gap between the screw threads and the bone. The thread profile also would affect the insertion torque and temperature. The AO cortical screw has a buttress thread while the IMEX pin has a standard V thread pattern.

The results of this study suggest that HA plasma sprayed coated screws may be inserted safely in equine MC3 bones.

CHAPTER 3
AN IN VIVO ANALYSIS OF OSSEOINTEGRATIVE PROPERTIES AND STABILITY OF HYDROXYAPATITE COATED AND UNCOATED 5.5 MM AO CORTICAL SCREWS IN EQUINE THIRD METACARPAL BONES

3.1. 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 (Bischofsberger et al., 2009). This suggests that the weak link of internal fixation in horses is the bone-screw interface (BSI). Bone resorption and screw loosening is most commonly the result of cyclic mechanical loading during limb use. 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 tissue (Zacharias et al., 2007). Hydroxyapatite coating of external fixation pins have been used to prevent pin loosening in humans by the promotion of osseointegration, the process of bone ingrowth into the HA coating (Aro et al., 1995; Magyar et al., 1997). The authors are unaware of any studies that have evaluated the use of hydroxyapatite coated cortical screws in the equine MC3 bone.

The objective of this in vivo study was to determine if the plasma sprayed hydroxyapapite coating of the cortical screws inserted unicortically in the dorsal diaphysis of the equine MC3 would promote osseointegration. This would be determined by comparing the mean extraction torque of the HA coated cortical screws with the mean insertion torque. A second objective is to measure osseointegration over time by comparing mean extraction torques of HA coated with uncoated cortical screws at four week intervals. 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 corresponding mean insertion torques after 4, 8, 12, and 16 weeks of

healing. We also hypothesized that mean insertion torques of the uncoated 5.5 mm cortical screws inserted in equine MC3 bones would be significantly greater than the corresponding mean extraction torques after 4, 8, 12, and 16 weeks of healing.

3.2. Materials and Methods

Four adult Thoroughbred horses between 2 – 7 years of age, free of orthopedic disease, were included in the study group. The investigation protocol was approved by the Institutional Animal Care and Use Committee. The animals were housed in single, 3 m x 3 m box stalls throughout the study period and horses were allowed access to free choice water and grass hay and supplemented with 8 pounds of a complete pelleted diet (Equine Senior™, Purina Mills, St. Louis, MO) per day, divided in 2 feedings. In the initial postoperative period (14 days), the horses were stall confined. Thereafter, the animals were allowed daily turnout in small paddocks. 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 screw implantation. An intravenous catheter was placed in the jugular vein using aseptic technique and perioperative antibiotics consisting of potassium-penicillin (22.000 IU/kg IV) and gentamicin (6.6 mg/kg IV) administered 30 minutes prior to induction of general anesthesia. Analgesia was provided by preoperative administration of flunixin-meglumine (1.1 mg/kg IV) and continued for 72 hours postoperatively, once a day. 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 with approximately 2-3 % isoflurane and 100% oxygen in a semi closed circle system. The animals were placed in dorsal recumbency and the surgical site was routinely aseptically prepared and draped.

Sixteen 5.5 mm AO cortical screws (Synthes, Paoli, PA) each 26 mm long had a plasma sprayed HA coating applied to the threads with a mean thickness of 50.0 µm ± 10.0 (Orchid Bio-Coat Inc, Southfield, MI) (Fig. 3.1). Sixteen 5.5 mm AO cortical screws, each 26 mm long 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.

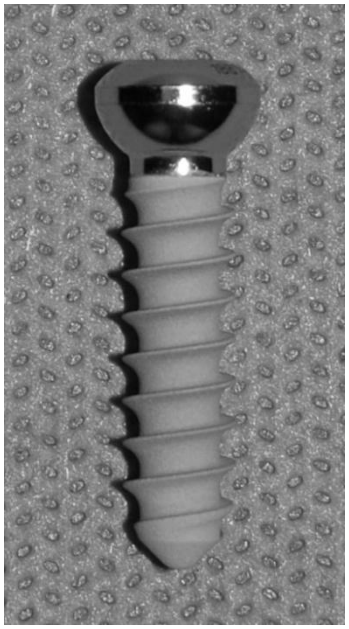

Figure 3.1. 5.5 mm AO cortical bone screw, HA plasma sprayed to a thickness of 50 ± 10 µm

For each limb, the mid-diaphyseal area was determined as the mid point between the carpometacarpal and metacarpophalangeal joint and four stab incisions were created in the dorsal mid diaphysis, using a 4-hole narrow dynamic compression plate (DCP) as a template. The holes were drilled perpendicular to and through the dorsal cortex of the MC3 bone using a 4.0 mm drill bit (Synthes, Paoli, PA). The depth of each hole was measured using a depth gauge and the thickness of the cis 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. The preassigned type of screw was inserted initially by hand using a screw driver and 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. Care was taken not to allow the head of the screw to engage the dorsal cortex. The insertion torques were recorded. The digital torque wrench has an accuracy of ± 2.5% clockwise rotation and ± 3.5% counterclockwise rotation with a resolution of 0.01 Nm. Drilling, tapping and screw placement were performed under constant irrigation using 0.9% NaCl solution (150 ml/ min). The stab incisions were closed using 2-0 polydiaxonone (PDS) in a cruciate pattern.

Light, protective bandages were applied to both limbs and the horse recovered from general anesthesia under assistance using head and tail ropes. Bandages were changed every other day until suture removal 12 days postoperatively and at each were time assessed for heat, swelling and discharge.

For each horse, the four screw locations (testing groups) of each limb were randomly assigned one of four numbers, 4, 8, 12 or 16 which corresponded to the plan of screw removal at 4, 8, 12 and 16 weeks post-operatively. Horses in this study were also included in a non-related study requiring general anesthesia at 4 weeks. For the first extraction torque measurements and screw removal, analgesia and anesthesia protocol were identical as described above and extraction torques were measured using a digital torque wrench with the horse in dorsal recumbency. At 8, 12 and 16 weeks the extraction torque measurements and screw removal was performed with the horses standing, sedated with a combination of detomidine (0.02 mg/ kg IV) and butorphanol tartrate (0.03 mg/ kg IV). Local anesthesia was provided by a combination of a high 4 point and dorsal ring block using a total volume of 15 ml of Lidocaine hydrochloride (2%). After each screw extraction, the incision site was closed with 2-0 PDS using a single

cruciate pattern and a sterile bandage applied. Bandages were maintained until suture removal 12 days post screw removal, and the horses were allowed daily turnout.

Mean ± standard deviation (SD) was calculated for the insertion and extraction torques for each screw type and each testing group. Paired samples were evaluated using *t*-tests for paired sample means within each testing group. Statistical significance was set at $p < 0.05$.

3.3. Results

For the screws assigned to extraction at 4, 8 and 12 weeks, there was no significant difference ($p = 0.34, 0.20, 0.79$, respectively) between the mean insertion torque for the HA-coated screws and the uncoated screws (Tables 3.1-3.3). For the screws assigned to extraction at 4, 8 and 12 weeks, there was no significant difference ($p = 0.68, 0.51, 0.89$, respectively) between the mean dorsal cortex thickness for the HA-coated screws and the uncoated screws (Tables 3.1-3.3).

At 4 weeks, there was no significant ($p = 0.12$) difference between mean extraction torque and the mean insertion torque of the HA coated screws (Table 3.1). The mean extraction torque of the uncoated screws was significantly ($p < 0.001$) less than the mean insertion torque of the uncoated screws (Table 3.1). The mean extraction torque for the HA coated screws were significantly ($p < 0.01$) greater than that of the uncoated screws (Table 3.1).

At 8 weeks, there was no significant difference ($p=0.07$) between the mean extraction torque of the HA coated screws and their mean insertion torque (Table 3.2). The mean extraction torque of the uncoated screws was significantly ($p < 0.027$) less than the mean insertion torque of the uncoated screws (Table 3.2). The mean extraction torque for the HA coated screws were significantly ($p < 0.008$) greater than that of the uncoated screws (Table 3.2)

At 12 weeks, the mean extraction torque of the HA coated screws was significantly ($p < 0.008$) greater than the mean insertion torque of the HA coated screws (Table 3.3). The mean extraction torque of the uncoated screws was significantly ($p < 0.036$) less than the mean insertion torque of the uncoated screws (Table 3.3). The mean extraction torque for the HA coated screws were significantly ($p < 0.0001$) greater than that of the uncoated screws (Table 3.3).

Clinical appearance

No signs of lameness were observed in the immediate postoperative period following screw insertion or extraction. Mild incisional seroma formation occurred in several horses which caused no discomfort and resolved without further treatment. At the end of the study period, excellent cosmetic results were achieved in all but one limb.

One of the uncoated screws could not be removed at 8 weeks and was left in place. The screw made several full turns and the extraction torque was measured before the screw driver stripped the hexagon recess in the screw head. Two HA coated screws could not be removed at 16 weeks and were initially left in place. In these screws, the screw driver stripped the hexagon recess in the screw head and did not allow measurement of the extract torque. Due to the inability to measure the extraction torques of two of the HA coated screws at 16 weeks, no statistical analysis was performed using the remaining data at 16 weeks.

One of the HA screws caused repeated suture line dehiscence and serous discharge. Local swelling and mild palpable discomfort was associated with this reaction. A screw removal device (Synthes, Paoli, PA) was used to extract the screw.

Suture dehiscence appeared again 10 days after the screw was removed and radiographic examination revealed a radiolucent area around the screw hole in the dorsal cortex.

Table 3.1 Mean ± SD of Screw Insertion and Extraction Torques And Dorsal Cortex Thickness for HA Coated and Uncoated screws at 4 Weeks.

Screw Type	Cortical Bone Thickness (mm)	Insertion Torque (N-m)	Extraction Torque (N-m)
Uncoated AO	20.8 ± 2.5[1]	5.45 ± 0.73[2,4]	2.65 ± 0.58[2,5]
HA Coated AO	21.5 ± 2.1[1]	6.06 ± 1.07[3,4]	4.76 ± 0.91[3,5]

[1] There is no significant ($p = 0.68$) difference between the mean cortical bone thickness for the HA coated and uncoated screws.

[2] The mean insertion torque is significantly ($p < 0.0011$) greater than the mean extraction torque for the uncoated screws.

[3] There is no significant ($p = 0.12$) difference between the mean insertion and extraction torques for the HA coated screws.

[4] There is no significant ($p = 0.34$) difference between mean insertion torques for the HA coated and uncoated screws.

[5] The mean extraction torque for the HA coated screws is significantly ($p < 0.011$) greater than that for the uncoated screws.

Table 3.2 Mean ± SD of Screw Insertion and Extraction Torques And Dorsal Cortex Thickness for HA Coated and Uncoated screws at 8 Weeks.

Screw Type	Cortical Bone Thickness (mm)	Insertion Torque (N-m)	Extraction Torque (N-m)
Uncoated AO	23.0 ± 2.7[1]	5.11 ± 0.58[2,4]	1.97 ± 1.67[2,5]
HA Coated AO	21.8 ± 2.1[1]	4.40 ± 0.79[3,4]	6.32 ± 1.49[3,5]

[1] There is no significant ($p = 0.51$) difference between the mean cortical bone thickness for the HA coated and uncoated screws.

[2] The mean insertion torque is significantly ($p < 0.0027$) greater than the mean extraction torque for the uncoated screws.

[3] There is no significant difference ($p=0.07$) between the insertion and the insertion torque for the HA coated screws.

[4] There is no significant ($p = 0.20$) difference between mean insertion torques for the HA coated and uncoated screws.

[5] The mean extraction torque for the HA coated screws is significantly ($p < 0.008$) greater than that for the uncoated screws.

Table 3.3 Mean ± SD of Screw Insertion and Extraction Torques and Dorsal Cortex Thickness for HA Coated and Uncoated screws at 12 Weeks.

Screw Type	Cortical Bone Thickness (mm)	Insertion Torque (N-m)	Extraction Torque (N-m)
Uncoated AO	21.5 ± 1.3[1]	4.78 ± 1.31[2,4]	2.45 ± 1.10[2,5]
HA Coated AO	21.3 ± 2.5[1]	5.10 ± 1.93[3,4]	9.93 ± 1.21[3,5]

[1] There is no significant ($p = 0.89$) difference between the mean cortical bone thickness for the HA coated and uncoated screws.

[2] The mean insertion torque is significantly ($p < 0.0036$) greater than the mean extraction torque for the uncoated screws.

[3] The mean extraction torque is significantly ($p < 0.008$) greater than the mean insertion torque for the HA coated screws.

[4] There is no significant ($p = 0.79$) difference between mean insertion torques for the HA coated and uncoated screws.

[5] The mean extraction torque for the HA coated screws is significantly ($p < 0.0001$) greater than that for the uncoated screws.

The standing horse was sedated with detomidine (0.02 mg/ kg IV) and butorphanol tartrate (0.03 mg/ kg IV). Local anesthesia was provided by a combination of a high 4 point and dorsal ring block using a total volume of 15 ml of Lidocaine hydrochloride (2%). Necrotic bone was debrided around the screw hole and the skin edges apposed primarily. The wound healed without further complication and the horse remained comfortable.

3.4. Discussion

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). Reducing the amount of motion of the screw during the immediate postoperative period can decrease screw failure and lead to bony incorporation of the implant (Soballe et al., 1993). Histological studies have shown that immediately after its insertion, a limited area of contact between screw threads and bone exists (Uhthoff, 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 (Uhthoff, 1973). The contact between the horizontal thread surface and bone is partly caused by tightening the screw to the plate or the cis cortex, in the case of lag screw fixation. In tightening the screw, the horizontal surface compresses the bone while the oblique under-surface of the thread, which is oriented towards the tip of the screw, is lifted away from the bone (Uhthoff, 1973). 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 (3.0 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 tap often cuts threads in the bone whose diameter is larger than the outer thread diameter of the screw, which results in an even smaller area of contact between the screw thread and bone, with the crest

of the screw threads losing contact with the bone. 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 (Uhthoff, 1973) which potentially can lead to fibrous integration of the implant under these unstable conditions (Clary and Roe, 1995).

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 and increases the chances for osseointegration (Soballe et al., 1993). 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 2.7 fold increase in the number of cycles to failure compared to the fixation secured with standard cortical screws (Sod et al., 2007). In a recent study, a 4.5 mm broad LC-DCP fixation of an osteotomized equine MC3 bone secured with plasma spray hydroxyapatite (HA) coated cortical screws was superior to uncoated cortical screws in resisting static overload forces and cyclic fatigue (Durham et al., 2010). These in vitro results suggest that HA coated screws improved the initial construct stability (Durham et al., 2010).

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 pin stability and osseointegration. 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 (Clary and Roe, 1995). In the case of the uncoated screws, there was approximately a 50% decrease in the mean extraction torques compared to the mean insertion torques at 4, 8 and 12 weeks. This suggests that osseointegration did not take place during the 12 weeks period. An increase in extraction torque compared to the insertion torque suggests that osseointegration has taken place during the given time period (Clary and Roe, 1995). In the case

of the HA coated screws there was no significant difference between the mean extraction and mean insertion torques at 4 weeks, but there was a 24% and 95% increase in the mean extraction torques compared to the mean insertion torques at 8 and 12 weeks, respectively. This suggests that osseointegration was occurring between 4 and 8 weeks and became evident by 8 weeks.

During the insertion of the HA coated and uncoated screws, the insertion torque initially continuously increased with each rotation as the threads of the screw engaged more bone of the dorsal cortex. Once the screw threads engaged all of the threads cut in the bone of the dorsal cortex, the insertion torque began to decrease to a plateau value which remained constant until just prior to the screw head coming into contact with the dorsal cortex. The plateau value at this point was recorded as the insertion torque. This provides an explanation for the determination that there was no significant difference between the mean insertion torque for the HA-coated screws and the uncoated screws.

The authors are unaware of any studies that have compared extraction torques of cortical screws removed from an equine long bone where the limb is loaded (weight bearing) with the limb unloaded (non-weight bearing). Potentially loading the limbs during screw removal could have resulted in increased compressive forces on the Haversian canals in the dorsal cortex resulting in increased frictional forces on both the HA coated and uncoated screws and hence increased extractional forces. However, the mean extraction torques for the uncoated screws was approximately 50% less than the mean insertion torques at weeks 4, 8 and 12 suggests that the effect of loading the limb during screw removal would be small.

Unicortical screw placement was chosen in this study in order to allow utilization of a single length 5.5 mm cortical screw. A 26 mm long 5.5 mm cortical screw was chosen so the screw threads, but not the screw head, would engage the dorsal cortex without contacting the

palmar cortex. In a typical clinical setting cortical screws are inserted bicortically and inserting the screws unicortically created a less stable model than had the screws been inserted bicortically.

Larger and stronger plates, screws and other internal fixation devices help reduce the incidence of catastrophic implant failure (Ruedi et al., 2007). With this principle in mind two plates have been recently introduced for the large animal patient, the 5.5 mm broad limited-contact dynamic compression plate (LC-DCP) and the 5.5 mm broad locking compression plate (LCP) (Synthes Vet, Paoli, PA). The increased size of these plates places the incision under great tension, thereby, potentiating postoperative necrosis and dehiscence (Bramlage, 1983; Turner, 1982). 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 (Bischofsberger et al. 2009). The weak link of internal fixation in the horses is the BSI (Bischofsberger 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 tranfixation pin cast (both traditional and tapered-sleeve) or a taper-sleeve transcortical pin external skeletal fixation device for the stabilization of distal radial, MC3 and MT3, and phalangeal fractures (Elce et al., 2006; Zacharias et al., 2007; Nunamaker and Nash, 2008). For these external fixation devices, pin loosening poses a significant problem for the equine patient (Zacharias et al., 2007), as well as for the human patient (Clary and Roe, 1995; Magyar et al., 1997), and the use of HA coated transfixation pins to improve stability at the bone-

pin interface has been the focus of several studies (Soballe et al., 1993; Clary and Roe, 1995; Magyar et al., 1997; Zacharias et al., 2007).

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 osseointegration occurs) and during the later healing period (after osseointegration occurs).

CHAPTER 4
FINAL DISCUSSION AND CONCLUSIONS

4.1. Summary

In summary we have compared 5.5 mm AO cortical bone screws with and without plasma-sprayed coating to a thickness of 50 ± 10 µm and their insertion properties as well as their performance in equine cortical bone over time.

We have determined that HA coated 5.5 mm cortical screws can safely been inserted in equine cortical bone. Given the geometry and size of the 5.5 tap and the thickness of the HA coating, no detrimental temperatures where achieved at any time point. Significantly higher insertional torque *in vitro* indicates better initial stability. The recent results by Durham et al. (2010) using the same type of screws *in vitro* prove that the HA coating provides significantly better stability than the regular stainless steel cortical screws. Better initial stability renders better resistance against the immense one time loads during recovery from anesthesia and the need for immediate weight bearing in the equine patient.

The *in vivo* results indicate a clearly superior osseointegration over time. In the recent review for equine metacarpal and metatarsal fractures by Bischofsberger (2009) only 32% of the cases had a positive outcome with only 25% of the horses returning to sound performance. Screw failure was a significant contribution for failure of the repair. The screws' contribution to stability has long been recognized in human orthopedics and different coatings are available for almost any long-term application of implantable biomaterials (Moroni et al., 1996 and 2003).

As some problems were encountered with the removal of the HA coated screws more long-term studies will be needed to establish the ease of removal in case that might become necessary in a clinical case. On the other hand, different currently used equine implants can not be removed by design (i.e. intramedullary nails, DCS/ DHS implants) and find their successful application due to clinical necessity. HA coated screws might become the implants of choice

wherever the removal is not critical or where the need for initial and increased long-term stability outweighs the unlikely implant removal.

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VITA

Timm Hilmar Gudehus was born in Langenfeld, Germany, in 1979. After moving to the small town of Coburg, being around horses was affordable and made up for most of his free time. Spending almost 20 years around horses, as an amateur as well as a professional horse trainer, it was clear that making a living had to depend on being around the equine world. He graduated from the Tierärztliche Fakultät of the Ludwig-Maximilians-Universität, München, Germany, in 2003 and financed his doctoral dissertation with a position in technical service for the Merial GmbH in Munich as well as fulfilling an internship close to the thoroughbred racetrack in a full service privately owned equine hospital in Munich. He graduated from the doctoral program with the academic title "Dr.med.vet.," honored by the aforementioned university with the official graduation in 2006. As his doctoral research was focused on orthopedic research in the area of implantology, it was obvious that an academic residency would be the optimal combination of orthopedic research and a surgical education in his field of expertise, the equine industry. In order to fulfill the necessary requirements for application to such programs, Timm was accepted for an Internship under board certified supervision with Drs. Rantanen and Martinelli at California Equine Orthopedics in San Marcos, California, in 2006. Timm was accepted into his primary ranked position of choice, an Equine Surgery Residency at Louisiana State University, in July 2007. He instantly sought mentorship with his major professor Dr. Gary A. Sod, who supported him and his surgical and academic training with guidance throughout this program.