Influence of bone cements on bone-screw interfaces in the third metacarpal and third metatarsal bones of horses

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Objective—To compare biomechanical strength, interface quality, and effects of bone healing in bone-implant interfaces that were untreated or treated with calcium phosphate cement (Ca-cement), magnesium phosphate cement (Mg-cement), or polymethylmethacrylate (PMMA) in horses.

Animals—6 adult horses.

Procedures—4 screw holes were created (day 0) in each third meta-carpal and third metatarsal bone of 6 horses. In each bone, a unicortical screw was placed in each hole following application of Ca-cement, Mg-cement, PMMA, or no treatment (24 screw holes/treatment). Screws were inserted to 2.82 N m torque. Horses were euthanized and bones were harvested at day 5 (16 screw holes/treatment) or day 182 (8 screw holes/treatment). Radiography, biomechanical testing, histomorphometry, and micro–computed tomography were performed to characterize the bone-implant interfaces.

Results—Use of Mg-cement increased the peak torque to failure at bone-implant interfaces, compared with the effects of no treatment and Ca-cement, and increased interface toughness, compared with the effects of no treatment, Ca-cement, and PMMA. Histologically, there was 44% less Ca-cement and 69% less Mg-cement at the interfaces at day 182, compared with amounts present at day 5. Within screw threads, Ca-cement increased mineral density, compared with PMMA or no treatment. In the bone adjacent to the screw, Mg-cement increased mineral density, compared with PMMA or no treatment. One untreated and 1 Ca-cement–treated screw backed out after day 5.

Conclusions and Clinical Relevance—In horses, Mg-cement promoted bone-implant bonding and adjacent bone osteogenesis, which may reduce the risk of screw loosening. (*Am J Vet Res* 2009;70:xxx–xxx)

Implant loosening is commonly encountered in humans and other animals that undergo orthopedic surgery and results in compromised construct stability, decreased patient comfort, and additional expenses.[1–15] The holding power of an implant in bone is associated with multiple factors such as the mechanical and structural properties of the implant, mechanical and physical properties of the bone, placement of the implant, load

ABBREVIATIONS

Ca-cement	Calcium phosphate cement
CT	Computed tomography
MCIII	Third metacarpal
Mg-cement	Magnesium phosphate cement
MTIII	Third metatarsal
PMMA	Polymethylmethacrylate
ROI	Region of interest

distribution, and bone-implant integration.[7,12–14,16–19] Cyclic loading, infection, inflammatory reaction around the implant and subsequent bone resorption, micromotion-induced implant loosening, and fatigue failure at the bone-implant or bone-cement interface are other common causes of implant failure.[16,20–25] Depending on the surgical procedure, the incidence of failure varies. Horses are particularly prone to implant failure because of their active nature, slow bone healing (compared with that of dogs and humans), and large body size as well as the load and shear forces placed on the implant.[3] In small animals, screw loosening is the most common complication in triple pelvic osteotomies, tibial pla-

teau leveling osteotomies, fracture repairs, and total arthroplasties.[4–7,10,11,26]

Various implant surface configurations, coating methods, and biomaterials have been developed to improve integration between bones and implants.[24,27–30] An assortment of osteoinductive and osteoconductive materials has been used to fill bone defects and to anchor implants to bone.[23] To achieve this, a material should adhere implant to bone, tolerate and transfer loads on the implant to bone, promote bone healing, and be readily absorbed at a rate that allows adequate time for osseointegration.[28,31] The biomechanical properties of the filler material should resemble those of bone and should be resistant to fragmentation and wear debris formation.[24,32] Furthermore, the formulation should be easy to apply, should not cause thermal damage during the process of curing, and should be tolerated by the host.[25,33]

Polymethylmethacrylate is an acrylic bone cement, which has been used for plate luting[34] and total arthroplasties for almost 50 years.[24,35–37] Because PMMA is nonabsorbable, 2 interfaces will inevitably exist—1 between the implant and cement and another between the cement and bone. Wear particle formation, thermal necrosis from the curing process, and fractures within the cement layer are known complications associated with the use of PMMA and can lead to failure of the implant construct.[12,20,25,35,38,39]

Calcium phosphate cement was the first biodegradable bone cement to be made commercially available. It can tolerate high compressive strength, fill in gaps between implant and bone,[40–42] act as an osteoconductive medium,[43] and increase biomechanical strength of the bone-implant interface.[40,44,a] However, Ca-cement lacks any adhesive properties and has a long absorption time.[40,42,45,b] Recently, magnesium-based alloys have been evaluated as orthopedic biomaterials.[33,46–49,a,b] Magnesium is a lightweight metal, which has mechanical properties similar to those of bone.[50,51] In the body, magnesium is the fourth most common cation, and approximately half of the magnesium is stored in bone. The magnesium cation is responsible for mediating activation of adhesion molecules such as integrins, which affect bioadhesion and the genotype of osteogenic cells.[52–58] These observations are supported by results of recent studies,[33,49,a,b] which suggest that a novel Mg-cement has unique adhesive and osteoproliferative properties. Magnesium phosphate cement significantly increases extraction torque of screws, compared with findings for other cements in vitro; adheres bone to bone; and induces osteogenesis in vivo.[33,49,a,b]

The purpose of the study reported here was to compare biomechanical strength, interface quality, and bone healing in bone-implant interfaces that were untreated or treated with Ca-cement, Mg-cement, or PMMA in the MCIII and MTIII bones of horses. Outcome measurements were clinical variables, serial radiographic findings, screw extraction torque, and histomorphometric and micro-CT data. We hypothesized that Mg-cement would improve interface strength and quality and would be absorbed faster than Ca-cement.

Materials and Methods

Animals—Six clinically normal adult horses (age range, 2 to 27 years; weight range, 450 to 584 kg) were included in the study. There were 3 mares, 2 geldings, and a stallion of various breeds (3 Quarter Horses, 2 Standardbreds, and 1 American Saddlebred). All horses were housed in individual box stalls (5 X 3 m) and fed hay and water ad libitum for the duration of the study. The protocol for the study was approved by The Ohio State University Institutional Animal Care and Use Committee.

Experimental procedure—All 6 horses underwent a surgical procedure (day 0) to place 4 bone screws in each MCIII and MTIII bone. Before surgery, a tetanus toxoid and penicillin G procaine (22,000 U/kg, q 24 h) were administered IM and gentamicin (6.6 mg/kg, q 24 h) and phenylbutazone (4.4 mg/kg, q 24 h) were administered IV. Prior to induction of anesthesia, the horses were sedated with xylazine hydrochloride (1.1 mg/kg, IV). Anesthesia was induced with diazepam (0.1 mg/kg, IV) and ketamine hydrochloride (2.2 mg/kg, IV) and maintained with isoflurane vaporized in oxygen in a semiclosed system.

In each anesthetized horse, 8-cm dorsal skin incisions were created in both MCIII and both MTIII bones at the level of mid-diaphysis. Four unicortical screw holes were then drilled by use of a power drill and a 3.5-mm drill bit; holes were drilled through the dorsal cortex from distal to proximal in a linear fashion at 2-cm intervals. The holes were manually threaded by use of a 4.5-mm tap and flushed with physiologic saline (0.9% NaCl) solution to remove any bone dust. In each bone, each hole was assigned to receive a different treatment (Ca-cement, Mg-cement, PMMA, or no treatment [24 screw holes/treatment]). Untreated screws were applied first, followed by those treated with PMMA,[c] Ca-cement,[d] and Mg-cement,[e] respectively, in a controlled block design so that all treatments were rotated and placed at each position an equal number of times. Each cement material was mixed separately (according to the manufacturers' instructions), and 0.5 mL was injected into the designated hole by use of a curved tip syringe. The screws (4.5-mm 316L stainless-steel cortical bone screws[f]) were inserted immediately after cement application to a defined torque of 2.82 N m by use of a torque wrench.[g] After all screws had been inserted into the predrilled holes in all 4 limbs, any excessive cement surrounding each screw head was removed and the incisions were lavaged prior to closure. In each limb, the subcutaneous tissue layers were closed in a simple continuous pattern with 2-0 polyglactin 910 suture, followed by closure of the skin in a similar pattern with 2-0 nonabsorbable monofilament polypropylene suture. A sterile bandage was then applied to each limb, and the horse was allowed to recover. Sterile bandages were maintained until the horse was euthanatized at day 5 or for a period of 3 weeks. For pain control after surgery, each horse was administered a combination of acepromazine maleate (0.02 mg/kg, IM, q 6 h) and morphine sulfate (0.06 mg/kg, IM, q 6 h) during the first 24 hours postoperatively and phenylbutazone (4.4 mg/kg, PO, q 24 h) for 3 days postoperatively. Treatment with antimicrobials was continued for 5 days after surgery.

Four horses were euthanatized at day 5; these horses were administered xylazine (1.1 mg/kg, IV) followed by an IV injection of pentobarbital sodium.[h] Both MCIII

and both MTIII bones were harvested immediately from each horse after euthanasia for biomechanical testing and further processing. The remaining 2 horses were euthanatized by use of the same protocol at day 182. At day 154, those 2 horses were administered calcein[i] (20 mg/kg, IV) dissolved in 2% sodium bicarbonate solution via a catheter inserted in the left jugular vein[59] to assess active bone formation. Calcein administration was repeated at day 179, 3 days prior to euthanasia of the 2 horses.

Bone-cement materials—Two injectable biodegradable bone cements (Ca-cement[e] and Mg-cement[f]) and 1 injectable nonbiodegradable bone cement (PMMA[d]) that had similar handling characteristics were chosen for the experimental procedure. The PMMA product was the first FDA-approved bone-cement material and is still widely used for multiple purposes. The PMMA consisted of methylmethacrylate (75%), PMMA (15%), and barium sulfate (10%). The Ca-cement used in the study was a commercially available bone cement that consisted of a calcium phosphate powder mixed with a sodium phosphate solution, which hardens to a carbonated apatite in vivo. The Mg-cement had similar properties as those of the Ca-cement, but is not yet commercially available. The composition of this cement was monopotassium phosphate (54%), magnesium oxide (33%), tricalcium phosphate (9%), and dextrose (4%).

Clinical evaluation—For each horse, a physical examination was performed before surgery (baseline), twice daily for the first 5 days following surgery, once daily thereafter until day 14, and then weekly until termination of the study. Examined variables included rectal temperature, heart rate, respiratory rate, and gastrointestinal tract sounds. Surgical sites were evaluated at the time of bandage changes. Lameness during walking at day 5 and during walking and trotting at day 182 was graded on a scale of 0 to 5[60] (0 = no signs of lameness at any time; 1 = intermittent signs of lameness during trotting; 2 = consistent signs of lameness during trotting; 3 = consistent lameness present during trotting with a head nod; 4 = consistent lameness present during walking; and 5 = minimal to no weight bearing at any time).

Radiography—For each horse, lateromedial and dorsopalmar-plantar digital radiographic views[j] were obtained before day 0 to confirm that there were no bony abnormalities in the MCIII or MTIII bones and to evaluate the thickness of the dorsal aspects of the cortices of the MCIII and MTIII bones for screw selection. The greatest endosteal to periosteal distance was used to select the length of the screws. Lateromedial radiographic views were also taken at days 5 and 182. Implant integrity and position, periosteal reaction (present or absent), and increase in bone mineral density within the medullary canal (present or absent) were recorded (**Figure 1**).

Collection and processing of specimens—After euthanasia, the left MCIII and MTIII bones were collected for biomechanical testing and the right MCIII and MTIII bones were collected for histomorphometric analysis from 3 horses; the left bones were collected for histomorphometric analysis and the right bones were collected for biomechanical testing from the other 3 horses. Biomechanical testing was performed immediately after euthanasia. For histomorphometric analyses, the MCIII and MTIII bones were cut in half longitudinally after removing the skin and soft tissues; the presence or absence of cement in the medullary canal was noted. Specimens were then cut into sections (each containing 1 screw) and fixed in neutral-buffered 10% formalin followed by dehydration and infiltration involving increasing grades of ethanol and embedding medium[k] for a period of 5 weeks. After infiltration, the samples were polymerized with the embedding medium. Each polymerized block was then affixed to a slide, and a longitudinal section of the screw and adjacent bone was ground with a microgrinder[l] to a thickness of 50 μm. The remaining specimen in the block was retained for micro-CT.

Biomechanical testing—A servohydraulic materials testing system[m] was used to determine the extraction torque at a displacement rate of 1°/s until the bone-screw interface failed. The specimen was fitted into a custom-made mold, and a screwdriver was connected to the testing system and to the screw head in the exposed dorsal aspect of the MCIII or MTIII bone. A constant rate of rotation was applied to the screw head, and a continuous recording of the angle of displacement and torque (N mm) was recorded. Peak torque to failure (N mm) was obtained, and calculations from the load-deformation curve were made for energy absorbed
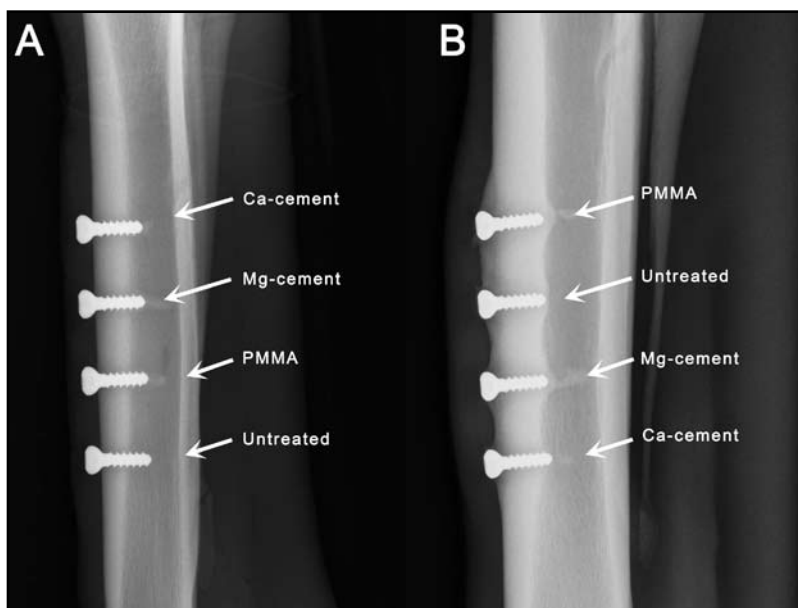

Figure 1—Representative digital radiographic images of MCIII (A) and MTIII (B) bones in 2 horses obtained at day 5 (A) and day 182 (B) after placement of 4 bone screws with application of Ca-cement, Mg-cement, PMMA, or no treatment. In the 6 study horses, screw hole treatments were placed at each position in the MCIII and MTIII bones an equal number of times. The presence of increased mineral density within the medullary canal and periosteal reaction were evaluated.
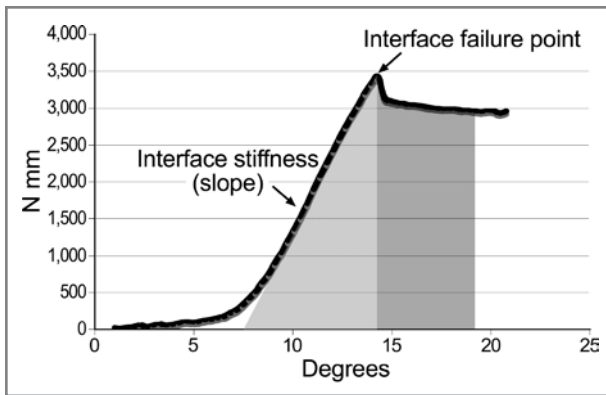
Figure 2—Representative load-deformation curve used for evaluation of the biomechanical properties of bone-screw interfaces in equine MCIII and MTIII bones in which 4 screws had been placed with application of Ca-cement, Mg-cement, PMMA, or no treatment. Peak torque to failure was recorded (N mm), and interface toughness (N mm•degree [light-gray shading]), postfailure extraction work (residual friction [N mm•degree {dark-gray shading}]), and interface stiffness (N mm) were calculated.

to failure (interface toughness), interface stiffness, and postfailure extraction work. Postfailure extraction work reflects the friction between the 2 surfaces at the failed interface; it was calculated as area under the curve for 5° after the point of failure (**Figure 2**).

Histomorphometric analysis—For all screw threads from the specimens harvested for histomorphometric analysis at days 5 and 182, the amount of cement within the screw thread was semiquantified (score 0 = no cement; 1 = 1% to 25% cement; 2 = 26% to 50% cement; 3 = 51% to 75% cement, and 4 = 76% to 100% cement). The characteristic appearance (homogeneous, heterogeneous, or presence of fissures) of the cement was recorded for all specimens from day 5. Bone-forming activity was quantified from the specimens collected at day 182 by use of point counting fluorescence labeling within each screw thread and in 3 bone zones adjacent to the screw by use of a microscope under fluorescent light at a wavelength of 400 nm. The assigned score equaled the number of labeled surfaces within each zone (**Figure 3**).

Micro-CT—Specimens collected at day 182 (n = 32 screw holes; 8 screw holes/treatment) were scanned longitudinally in 35-μm sections by use of micro-CT.[n] Prior to scanning, all screws were removed from the specimens with a screwdriver to prevent beam-hardening artifact from the metal implant. The remaining bone samples were scanned, and ROIs were selected from the bone between the screw threads and in the bone just adjacent to the screw thread (**Figure 4**). Mineral densities were recorded from the selected ROIs. The mineral density between the screw thread represented the mineral density of the remodeling bone and the mineral content of the remaining cement, whereas in the bone adjacent to the screw, only bone mineral density was measured. Densities were standardized for x-ray attenuation differences by use of a calibration phantom composed of a known concentration of hydroxyapatite embedded in lucite. A physical beam-hardening filter and a modified Feldkamp algorithm were used to reduce noise, and a
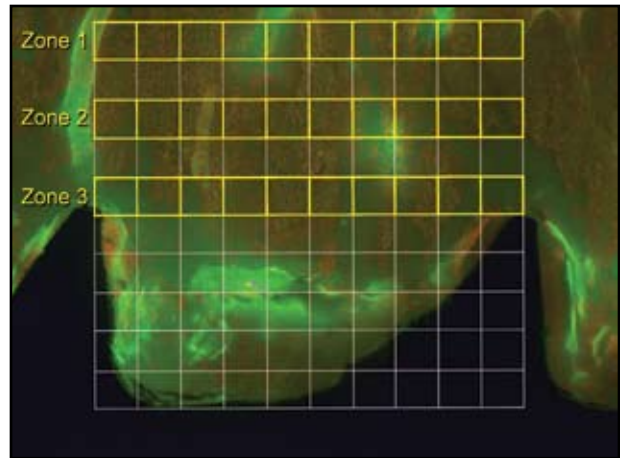

Figure 3—Representative photomicrograph of a bone-screw interface from an equine MTIII bone in which a screw was placed with application of PMMA 182 days earlier. The horse received calcein at days 154 and 182 (prior to euthanasia and harvesting of bone specimens). Bone-forming activity at screw locations was semiquantified from such specimens by point counting fluorescence labeling within each screw thread and in 3 bone zones adjacent to the screw during microscopic examination under fluorescent light at a wavelength of 400 nm. In this specimen, notice the autofluorescence of the PMMA and new bone formation adjacent to the screw thread.

multimodal 3-D imaging software program[o] was used to reconstruct images.

Statistical analysis—All data were analyzed by use of a statistical software program.[p] Objective data from the biomechanical testing, micro-CT, and assessments of bone-forming activity were analyzed with 1-factor (treatment) ANOVA and a Tukey multiple comparison test. Gaussian distribution was confirmed by use of the D'Agastino and Pearson omnibus normality test. Non-normally distributed data from mineral density calculations were logarithmically transformed prior to analysis. For the scored data (histomorphometric analyses), Kruskal-Wallis and Dunn multiple comparison tests were used to assess differences in the amount of cement present at days 5 and 182. A Mann-Whitney U test was used for the paired scored data. Differences were considered significant at a value of $P < 0.05$.

Results

Clinical evaluation—Physical examination findings were within reference limits for all horses during the initial 5 days after screw placement. By day 7, 1 horse had developed swelling in the distal aspect of the surgical site in both forelimbs, which persisted until termination of the study. No lameness (grade 0) was observed at day 5 while walking or day 182 while walking or trotting in any horse.

Radiographic evaluation—At day 5, all 96 screws were in position, as determined radiographically. At day 182, radiography revealed that 2 of the 32 screws in the bones of the remaining 2 horses had backed out of position. One screw had received no treatment, and the other screw had received treatment with Ca-cement; both of these screws were positioned in the most distal

hole in MCIII bones. Incidence for screw back-out at day 182 was 6.25%.

At day 182, periosteal reaction was present around the screw heads for 4 of 7 untreated screws, 6 of 7 screws that were treated with Ca-cement, and 6 of 8 screws that were treated with Mg-cement or PMMA. At day 5, greater mineral density from the presence of cement was observed in the medullary canal in 13 of 16 screws that were treated with Ca-cement, 14 of 16 screws that were treated with Mg-cement, and 15 of 16 screws that were treated with PMMA. At day 182, greater mineral density was observed in the medullary canal in 2 of 8 screws that were treated with Ca-cement, 7 of 8 screws that were treated with Mg-cement, and 6 of 8 screws that were treated with PMMA. With regard to Ca-cement–treated screws, radiographic evidence of increased mineral density in the medullary canal was observed significantly (P = 0.015) less frequently at day 182, compared with findings at day 5.

Biomechanical testing—Use of Mg-cement increased the peak torque to failure, compared with the effect of no treatment (P = 0.019) or Ca-cement (P = 0.012). Compared with the effect of PMMA, the use of Mg-cement similarly increased peak torque to failure, although the difference was not significant. Use of Mg-cement increased the interface toughness (energy absorbed to failure), compared with the effect of no treatment (P = 0.007), Ca-cement (P = 0.012), or PMMA (P = 0.027; **Table 1**; **Figure 5**). There were no significant differences among the treatment groups with regard to interface stiffness or postfailure extraction work. Also, there were no significant differences in biomechanical strength of the screws between the male and female horses. The interface failed consistently at the screw-cement interface for the Ca-cement, Mg-cement, and PMMA.

Histomorphometric analysis—Cements had a characteristic appearance at day 5. The Ca-cement was most often heterogeneous in appearance with several fissures and cracks within the cement material. The Mg-cement was more homogeneous but had a granular appearance. The PMMA appeared cellular and homogeneous. More than 90% of the threads in Ca-cement–, Mg-cement–, and PMMA-treated screws were filled with cement at day 5, and there was no difference (P > 0.05) in the cement score among those treatments. At day 182, there was significantly (P < 0.001) less Ca-cement and Mg-cement at the interface, compared with findings at day 5 (**Table 2**; **Figure 6**). Calcein label was detected with greater frequency in the screw threads than in the bone adjacent to the screws. Differences in bone-forming activity among treatments could not be detected after calcein labeling at days 154 and 182 (**Table 3**).

Micro-CT—Mineral density measurements were obtained for specimens collected at day 182 (Table 3). The Ca-cement increased the mineral density within the screw threads, compared with the effect of no treatment or PMMA (P < 0.001). The Mg-cement increased the mineral density within the screw threads, compared with the effect of PMMA (P < 0.001). The Mg-cement increased the mineral density of bone adjacent to the screw, compared with the effect of no treatment or PMMA (P = 0.008). The sex of the horses did not have an effect on the mineral density measurements.

Discussion

To our knowledge, this is the first study to compare the effects of a specific formulation of Mg-cement with a commercially available Ca-cement or PMMA in a bone-implant interface in vivo. The results of the present study supported the findings from previous investigations, which indicate that Mg-cement is a biocompatible bone cement that can considerably improve bone-implant interface bonding and induce osteogenesis
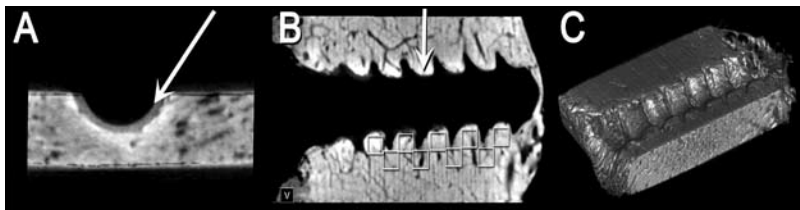

Figure 4—Micro-CT images of 3 representative specimens of equine MTIII bones at day 182 after screw placement to illustrate mineral density assessments. A—Transverse section of the screw hole. In the screw hole, there is a uniform layer of PMMA (arrow). B—Longitudinal section of the screw hole. Notice the material of high mineral density within the screw thread (arrow). The ROIs for calculations are illustrated (boxes). C—Three-dimensional reconstruction image of the bone-screw interface.

Table 1—Biomechanical properties of bone-screw interfaces in both MCIII and both MTIII bones of 4 horses 5 days after placement of 4 screws in each bone with application of Ca-cement, Mg-cement, PMMA, or no treatment (4 different screw hole treatments/bone).

Variable	No treatment	Ca-cement	Mg-cement	PMMA	P value*
Peak torque to failure (N mm)	1,701 ± 164	1,665 ± 148	2,383 ± 198[a]	1,981 ± 240	0.046
Stiffness (N mm)	334 ± 37	328 ± 59	420 ± 47	336 ± 40	0.460
Toughness (N mm•degree)	214 ± 27	205 ± 45	372 ± 37[a,b]	246 ± 35	0.011
Postfailure extraction work (N mm•degree)	127 ± 18	101 ± 27	185 ± 53	114 ± 32	0.364

Data are presented as mean ± SEM (based on 16 screws in each treatment group).
*A value of P < 0.05 indicates a significant difference for this variable among treatment groups.
[a]For this variable, the value for Mg-cement–treated screws is significantly different (P < 0.05) from the value for untreated and Ca-cement–treated screws. [b]For this variable, the value for Mg-cement–treated screws is significantly different (P < 0.05) from the value for PMMA-treated screws.

in adjacent bone.[34,50,a,b,q] In our study, biomechanical testing was conducted at day 5 after screw placement, at which time the increases in extraction torque and interface toughness were most likely attributable to the
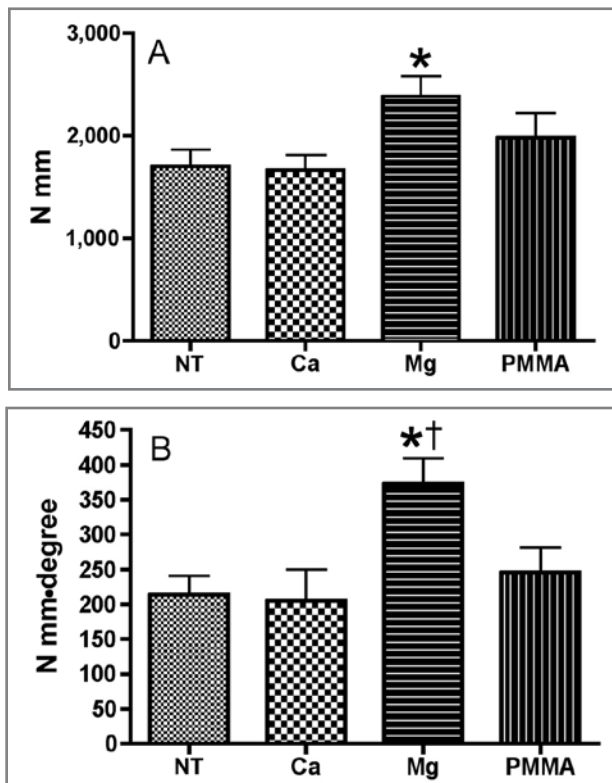

Figure 5—Mean ± SEM peak torque to failure (N mm; A) and interface toughness (N mm•degree; B) for screws that were inserted in equine MCIII and MTIII bones with application of Ca-cement, Mg-cement, PMMA, or no treatment (NT) 5 days earlier. *Value for Mg-cement–treated screws is significantly different ($P < 0.05$) from the value for untreated and Ca-cement–treated screws. †Value for Mg-cement–treated screws is significantly different ($P < 0.05$) from the value for PMMA-treated screws.

adhesive properties of the Mg-cement.[50,a,b] This effect is of particular clinical value because implant loosening commonly occurs during the early postoperative period.[5–7] Indeed, in our study, 2 screws backed out of their locations after day 5 and probably at day 7 when swelling over the screws was detected. There was no difference in the postfailure extraction work among the treatment groups, which can be explained by the gross and micro-CT observations that the interface failed consistently between the screw and the cement.

Radiography performed in 2 horses at day 182 revealed that screw back-out occurred at the most distal screw hole in 2 forelimbs. No signs of infection were evident histologically, and the failures most likely were a result of cyclic loading. One of the failed screws had received no treatment, and the other had been treated with Ca-cement. This clinical observation relates to the results from the biomechanical testing, which indicated that Mg-cement and PMMA provided better interface stability.

At day 182 after screw placement, radiography revealed that the density of the medullary canal was increased more often after application of Mg-cement than it was after application of Ca-cement. Results of histomorphometric analysis indicated that there was significantly less cement at the screw interface in Mg-cement–treated screws, compared with the amount of cement at the screw interface in Ca-cement–treated screws. This increased density may therefore represent a greater bulk of cement material that is slow to be absorbed or increased osteogenesis (ie, bone density) adjacent to the cement, as observed adjacent to the screw in the cortex. Similar observations have been reported in studies[42,45] to evaluate absorption of various types of Ca-cements. Differences in curing time and flow characteristics may have contributed to the presence of more Mg-cement than Ca-cement in the medullary canal. Importantly, for comparison of mechanical properties, all screws had a similar amount of cement within their threads.

Table 2—Histomorphometric measurements at bone-screw interfaces in both MCIII and both MTIII bones of 6 horses at 5 and 182 days after placement of 4 screws in each bone with application of Ca-cement, Mg-cement, PMMA, or no treatment (4 different screw hole treatments/bone).

Time point	Variable	No treatment	Ca-cement	Mg-cement	PMMA	P value*
Day 5	No. of screw threads evaluated	55	51		51	47
	No. of screw threads with cement (%)	0 (0)	47 (92.2)	50 (98.0)	47 (100)	0.08
	Cement score†	N/A	3.2 ± 1.2	3.6 ± 1.2	3.4 ± 1.2	0.008
	Cement characteristic (%)					
	Homogeneous	N/A	44.68	54	53.19	0.60
	Heterogeneous	N/A	55.32	46	46.81	0.60
	Fissures	N/A	59.57	4.0	2.13	< 0.001
Day 182	No. of screw threads evaluated	83	78		86	75
	No. of screw threads with cement (%)	0 (0)	37 (47.4)	28 (32.6)ᵃ	68 (90.7)	< 0.001
	Cement score†	N/A	1.8 ± 1.3	1.1 ± 1.0ᵃ	3.4 ± 1.2	< 0.001
	Bone activity score‡	5.0 ± 2.5	4.9 ± 2.4	4.2 ± 2.8	4.2 ± 2.7	0.38

Data are presented as mean or mean ± SD (based on 16 screws in each treatment group [4 horses] at day 5 and 8 screws in each treatment group [2 horses] at day 182).
†The amount of cement within the screw threads was semiquantified (score 0 = no cement; 1 = 1% to 25% cement; 2 = 26% to 50% cement; 3 = 51% to 75% cement, and 4 = 76% to 100% cement). ‡Bone activity score (0 to 10) reflects the number of fluorescein labeled surfaces within the screw thread and in 3 zones adjacent to the screw thread.
ᵃFor this variable, the value for Mg-cement–treated screws is significantly different from the value for Ca-cement–treated ($P = 0.01$) and PMMA-treated ($P < 0.001$) screws.
NA = Not applicable.
See Table 1 for remainder of key.

The PMMA was detected frequently in the medullary canal at day 182, as would be expected following use of a nonabsorbable material. At that time point, Ca-cement was identified in the medullary canal significantly less frequently. It has been reported[34] that Ca-cement is easily flushed away from the surgery site, and in the present study, it is possible that bleeding and positioning of the limbs during surgery can reduce the amount of Ca-cement retained within the medullary canal at the screw sites.

Both the biodegradable Ca- and Mg-cements used in the present study were partially absorbed at day 182; however, the absorption of Ca-cement was significantly slower than that of Mg-cement, based on the quantity remaining at the interfaces. Absorption of Ca-cements can be prolonged, and incomplete absorption has been detected as long as 78 weeks after implantation.[45] In horses, Mg-cement is not absorbed after 7 weeks,[33] but in the distal portion of the femur of rabbits, 63.6% is absorbed after 12 weeks and 83.8% is absorbed after 26 weeks.[r] In that same study in rabbits, absorption of a Ca-cement was significantly slower: 37.4% was absorbed after 12 weeks, and 61.8% was absorbed after 26 weeks. Results of our study further support that absorption of Mg-cement is more rapid than absorption of Ca-cement.

In the present study, calcein was administered to 2 horses at days 154 and 182, but there was no difference in bone-forming activity among the Ca-cement, Mg-cement, and PMMA, despite histomorphometric evidence that bone formation occurred. Specifically, the Mg-cement increased density of the bone adjacent to the screw, compared with findings in untreated screws or screws treated with PMMA. On the basis of an osteoproliferative effect of magnesium-based alloys detected in other studies,[33,c] the increased density of the bone adjacent to Mg-cement–treated screws was likely a result of increased bone-forming activity that occurred earlier than day 154. Injection of calcein earlier in the phase of bone healing may be necessary to improve our understanding of bone activity.

At day 182 after screw placement, bone density within the screw threads was greatest for the Ca-cement–treated screws. Bone density within the screw threads was significantly greater for Ca-cement–treated screws and Mg-cement–treated screws, compared with the effect of PMMA. Importantly, this density measurement reflects a composite of the cement and bone that has replaced the cement that was undergoing absorption. Histomorphometrically, there was more Ca-cement than Mg-cement within the screw threads, which probably accounts for the higher bone density. At day 182, Mg-cement was absorbed to a greater extent than was Ca-cement; therefore, the micro-CT measurement reflects more newly woven bone.[61] An increase in bone mineral density in the bone adjacent to the screw was associated only with the use of Mg-cement. This may support the osteogenic properties of the Mg-cement reported previously.[33,62–64] At day 182, PMMA had the lowest values for bone mineral density within the screw thread, which reflects the presence of cement that does not contain mineral. The sex of the horses did not have an apparent influence on the bone mineral density measurement; however, the number of animals included in the present study was small. Nevertheless, a recent study in 15 horses by Fürst et al[65] revealed that neither sex nor age affected bone mineral density measurements.

Overall, the procedures performed in the present study proved to be useful for evaluating the properties of implant interfaces, specifically the screw-cement and cement-bone interfaces. The midportion of the MCIII and MTIII bones in horses has a region of relatively uniform cortical thickness and density, which permits placement of multiple screws in each bone and allows each bone to
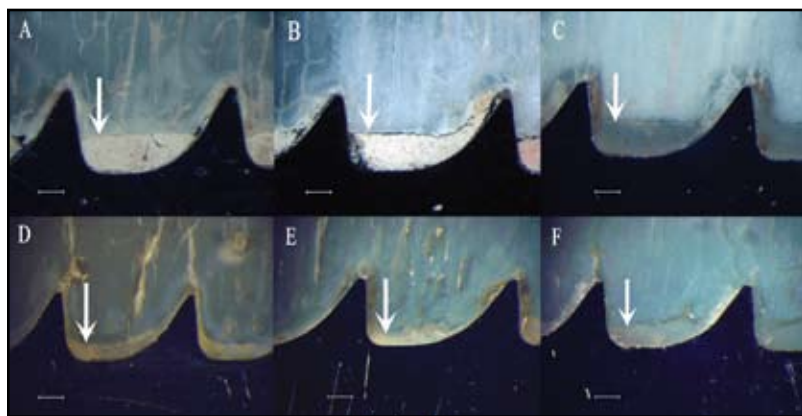

Figure 6—Photomicrographs of representative longitudinal sections of screws that were placed 5 days (A–C) and 182 days (D–F) earlier in equine MCIII and MTIII bones with application of Ca-cement (A and D), Mg-cement (B and E), or PMMA (C and F). Arrows indicate the surface of the cement or PMMA. Notice that the extent of filling with Ca-cement, Mg-cement, or PMMA is equivalent at day 5 and that partial absorption of Ca- and Mg-cement has occurred by day 182. In all panels, bar = 400 μm.

Table 3—Mineral density measurements (Hounsfield units) determined via micro-CT in both MCIII and both MTIII bones of 2 horses at 182 days after placement of 4 screws in each bone with application of Ca-cement, Mg-cement, PMMA, or no treatment (4 different screw hole treatments/bone).

ROI	No treatment	Ca-cement	Mg-cement	PMMA	P value*
Screw thread	3,373 ± 465	3,711 ± 501[a]	3,644 ± 421[b]	3,249 ± 520	< 0.001
Bone adjacent to the screw hole	3,145 ± 524	3,357 ± 587	3,418 ± 420[a]	3,151 ± 503	< 0.001

Data are presented as mean ± SD (based on 8 screws in each treatment group).
[a]For this variable, the value for this treatment group is significantly greater than the values for the untreated and PMMA-treated screws.
See Table 1 for remainder of key.

serve as its own control specimen. Allocation of each treatment to a different screw hole in each limb of each horse further ensured a similar testing environment for all treatments. Extraction torque measurements were readily performed, and the method used was a reliable means by which the mechanical stability of the interface could be characterized. Commonly, biomechanical testing of screws is performed by use of axial pullout tests.[66–69] A pullout test biomechanically evaluates the holding power of the material (bone or cement) surrounding the screw.[14,67–69] Extraction torque more likely reflects the strength and bonding characteristics of the interface as well as the resistance to cyclic forces driving the screw to back out, which is the mechanism of failure most commonly observed in clinical situations.

The cement materials used in the present study had different handling characteristics that were relevant to clinical application. Both the Ca- and Mg-cements were easy to mix and inject through the tip of the syringe; for both products, the study procedures could be completed (within a period of approx 10 minutes) before hardening commenced. The PMMA was readily mixed and injected but emitted noxious fumes and began to harden faster than the Ca- and Mg-cements. The Ca-cement was easy to inject, whereas the Mg-cement occasionally clogged the injection syringe tip as a result of its mildly granular texture. This could be remedied by use of a needle to unblock the tip of the syringe. In all instances in the present study, Ca-cement, Mg-cement, and PMMA were applied correctly in the assigned holes and even distribution of cement around each screw was confirmed histomorphometrically.

The results of the present study indicated that the Mg-cement possessed several beneficial characteristics of a biological fixator. In the early postoperative period, Mg-cement improved bone-implant stability and most of the cement was absorbed in a period comparable to bone healing (approx 26 weeks). Woven bone replaced the Mg-cement, thereby recreating a bone-screw interface. The potential osteogenic properties in the bone adjacent to the Mg-cement–treated screws should be further explored and may be of benefit in the healing of bone. Both Ca- and Mg-cements had good handling and injection characteristics, but a clinical benefit with the use of Ca-cement could not be demonstrated. The Mg-cement has the potential to improve the outcome in orthopedic surgeries through these beneficial properties.

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b. Bertone AL, Hackett B, Litsky A, et al. A magnesium injectable formulation adheres bone to bone and tendon to tendon (abstr), in *Proceedings*. 51st Annu Meet Orthop Res Soc 2005;1007.
c. Simplex P, Stryker Inc, Kalamazoo, Mich.
d. Norian skeletal repair system, Synthes, Paoli, Pa.
e. Osteocrete, Bone Solutions Inc, Dallas, Tex.
f. AO, Synthes, Paoli, Calif.
g. UTICA TCI-150-3/8 torque wrench, All-Spec Industries, North Wilmington, NC.
h. Euthasol, Virbac AH, Fort Worth, Tex.
i. Calcein, Sigma-Aldrich, St Loius, Mo.
j. EKLIN Digital Radiography Medical System Inc, Sunnyvale, Calif.

k. Tecnovit 7100, Kulzer, Wehrheim, Germany.
l. Exact System Leica Microsystems Nussloch GmbH, Nussloch, Germany.
m. Bionix 858 Servohydraulic Biaxial Materials Testing System, MTS System Corp, Eden Prairie, Minn.
n. Inveon PET Module, Siemens Medical Solutions Inc, Malvern, Pa.
o. Inveon Research Workplace, Siemens Medical Solutions Inc, Malvern, Pa.
p. Prism, version 4.0a, Graph Pad Software Inc, San Diego, Calif.
q. Witte F. Degrading magnesium implants increase periosteal and endosteal bone formation (abstr), in *Proceedings*. 50th Annu Meet Orthop Res Soc 2004;page.
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