# Improved osseointeraction of calcium phosphate-coated external fixation pins

## Studies in calves

David E Anderson[1], Guy St-Jean[1], Daniel C Richardson[2], Richard M DeBowes[1], James K Roush[1], Stephen R Lowry[2], Phillip W Toll[2], Harold M Aberman[3], David C Van Sickle[4] and James J Hoskinson[1]

We investigated osseointeraction of solution-precipitated calcium phosphate (SPCP)-coated transfixation pins used in external skeletal fixation of a calf stable fracture model. One group (SPCP) received centrally-threaded transfixation pins which had SPCP coating; the other group (control) received identical, but not coated, pins. Radiographs were obtained 1 and 40 days after surgery and examined for evidence of osteolysis. Bone phase $^{99m}$Tc-MDP studies were performed 6 and 28 days after surgery. Calves were killed 40 days after surgery and mechanical tests performed. Dual-energy x-ray absorptiometry (DEXA) and histomorphometric analyses were done. A smaller proportion of SPCP pins (5/24) had evidence of discharge during the study compared with control pins (21/24). A smaller proportion of SPCP pins (4%) had radiographic evidence of osteolysis compared with control pins (42%). Uptake of $^{99m}$Tc-MDP was similar for SPCP and control calves. Uptake was significantly greater in bone segments showing radiographic evidence of osteolysis than in bone segments not having osteolysis. Yield stress (MPa) for axial displacement was similar in the treatment groups. Bone mineral density was less in SPCP pins. Affinity index and interface histologic score were greater and osteoclastic index less in SPCP calves. Coating of transfixation pins with solution-precipitated calcium phosphate improved the osseointeraction of pin and bone during this 40-day study.

[1]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, [2]Mark Morris Institute, Topeka, Kansas, [3]Howmedica Inc., Pfizer Hospital Products Group, Rutherford, NJ, [4]Department of Basic Medical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN, USA.
Correspondence: Dr. David Anderson, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, Ohio, USA, 43210. Tel +1 614 292-6661. Fax -0895.
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Hydroxyapatite ceramics have been used as coatings for prosthetic implants (Geesink 1990). These ceramics are biocompatible (van Blitterswijk et al. 1990) and osteoconductive (Cook et al. 1992, Ohgushi et al. 1993). Rapid bone-bonding has been demonstrated within 3 weeks of implantation (Burr et al. 1993). Ceramics are usually applied by plasma spray (Lemmons 1988). Plasma spray may result in uneven coating thickness and gaps if the metal surface is uneven or porous (Brown 1984). Solution-precipitation of calcium phosphate on metal substrates has recently been developed. This process results in a uniform layer of calcium phosphate crystals, similar in morphology to naturally-occurring hydroxyapatite.

Complications resulting from the use of transcortical external fixation pins may cause fixation failure or patient morbidity (Aro et al. 1993). Microcortical damage, thermal injury and cyclical mechanical forces (Matthews et al. 1984, Egger et al. 1986) cause osseous remodeling, resorption and increased porosity around the pin (Aro et al. 1993). Major and minor pin tract sepsis ranges from 0% in both categories to 20% and 80%, respectively (Green 1983). Chronic osteomyelitis after pin removal ranges from 0 to 4 % (Green and Ripley 1984). Pin tract infection present at pin removal has been associated with subsequent infection of internal fixation devices, despite a separation period of 65 days (Maurer et al. 1989).

We hypothesized that application of a solution-precipitated calcium phosphate (SPCP) coating to transcortical pins used in external fixation would result in rapid bone-bonding to the pins and improved osseointeraction of the implants. The aim of this study was to determine the effect of solution-precipitated calcium phosphate coating of transcortical pins on the bone-implant interface.

## Animals and methods

12 male Holstein calves, 10–14 days old and weighing between 34 and 45 kgs, were used. The study protocol was approved by the Institutional Laboratory Animal Care and Use Committee at Kansas State University.

Calves were randomly assigned to one of two treatment groups, using a balanced, incomplete block design. Group 1 calves (SPCP) received positive-profile, centrally threaded transfixation pins (Apex Transfixing Pin, HowMedica Inc, Rutherford, NJ, USA) in which the centrally threaded portion of all pins had a coating of solution-precipitated calcium phosphate (Constanz BR, Osaka GC. Hydroxyapatite Prosthesis Coatings. US Patent 5,164,187; Figure 1). Solution precipitation was used to coat calcium phosphate onto the surface of the centrally threaded region of the treatment pins providing a coating approximately 30 μm thick (Peri-apatite, Howmedica Inc, Rutherford, NJ, USA). Group 2 calves (control) received identical, but not coated, transfixation pins. Procaine penicillin G (22,000 U/kg, sc, Q12hr) was administered preoperatively and for 7 days after surgery to control for the effects of potential early postoperative sepsis on the bone-implant interface. Phenylbutazone (5 mg/kg, p.o., once) was administered, if required, to control immediate postoperative discomfort.

Each calf was anesthetized by administration of xylazine hydrochloride (0.04 mg/kg, IV) and halothane vaporized into oxygen via a face-mask. Then, orotracheal intubation was performed and the calf was maintained using halothane vaporized into oxygen in a semi-closed circle system. Each calf was placed in dorsal recumbency and the right forelimb was suspended. The right forelimb was clipped and aseptically prepared for surgery.

Placement of transcortical pins was standardized by using a pre-designed template attached to the lateral aspect of the right metacarpus by 3, 0.8 mm Kirschner wires. 2 transcortical pins were placed from lateral to medial through the proximal metacarpus 2 cm apart, and 2 pins were inserted through the distal metacarpus 2 cm apart. The 2 central pins were separated by 4 cm. Prior to each pin insertion, a 1-cm longitudinal skin incision was made and a 3.2 mm pilot hole was drilled through the metacarpus. Transfixation pins were 4 mm diameter 316L stainless steel pins, with a cutting tip and a 5 cm long, centrally located, self-tapping, 5 mm diameter threaded region. The template was removed and the pins were connected by two 8 mm diameter aluminum side bars. A 5 cm long incision was made between the two central pins, immediately dorsal to the lateral digital extensor tendon. A transverse osteotomy was made, using a pneumatic saw, midway between the central pins. The skin was closed with #2-0 nylon suture in an interrupted cruciate pattern and a bandage was applied to the limb.

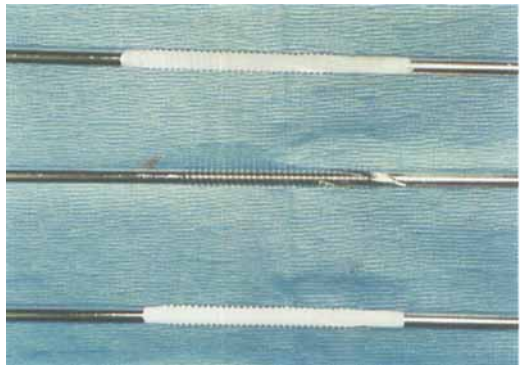

Figure 1. Solution-precipitated calcium phosphate-coated transfixation pins (thread diameter 5.0 mm, core diameter 4.0 mm). The center pin is not coated.

All calves were housed individually. Bandages were removed and the limb examined daily for 5 days after surgery. Then the bandages were changed every 3 days for the remainder of the study. Each site of insertion and exit of the transcortical pins was inspected for evidence of drainage or odor. The limbs were evaluated for swelling, redness or pain. The sidebars and clamps were examined for loosening, instability or bending. Statistical comparisons were performed for pins with discharge and the number of calves affected in each treatment group.

High-detail dorsopalmar and lateromedial radiographic images were obtained 1 day and 40 days after surgery. Radiographs were evaluated for pin position and the insertion cortex and exit cortex for each pin were evaluated for radiographic evidence of osteolysis. Statistical comparisons were performed for pins with osteolysis and the number of calves affected with osteolysis in each treatment group.

Nuclear scintimetry was performed at means of 6 and 28 days after surgery in all calves. Radioisotope uptake for bone phase scintimetry was performed 2 hours after administration of $^{99m}$Tc-MDP. The metacarpus of each treatment limb was analyzed by determining scintillation counts in the proximal and distal bone segments, excluding the osteotomy. The contralateral metacarpus was analyzed and used as an internal control against physiologic variability between calves. The ratio of the region of interest to the internal control (gamma count ratio) was used for statistical analysis.

All calves were killed approximately 40 days after

surgery. The right metacarpus was collected and each end of the bone embedded in PMMA blocks (Technovit, Jorgensen Laboratories, Loveland, CO, USA). The limb was attached to a testing frame and resistance to axial displacement (push-out test) was determined for all pins using a servohydraulic testing machine (Instron Model 4201, Instron Corporation, Canton, MA, USA). Displacement force was applied at a constant rate, 25 mm/min, until yield stress was reached. Displacement tests were stopped at the yield point (immediately after peak resistance was achieved and plateau observed) to minimize disruption (plastic deformation) of the specimens so that a histologic examination of the test specimens could be done. Mechanical data were recorded for each pin. The 4 push-out tests were averaged for each calf and the mean was used for statistical comparisons. Then bone segments containing each pin and extending 1 cm proximal and distal to the pin were collected. Each bone segment was placed in 10% buffered formalin solution and stored.

60 days after collection, dual-energy x-ray absorptiometry (DEXA) was performed on each bone segment, using standard techniques including a metal subtraction program (Hologic QDR-2000, Hologic, Waltham, MA, USA). Subregional analysis of bone was performed for bone 5 mm proximal and distal to the implant. Bone mineral density ($g/cm^2$) was determined for each pin subregion. The BMD was averaged for each calf, and the mean was used for statistical comparisons. Then bone segments were washed and stored in 70% ethanol solution.

Slides for histomorphometric evaluation were made on 12 undecalcified bone segments with the metal implant in situ. Segments were randomly selected from each group, so that the 4 pin sites were equally represented. Bone segments were dehydrated by incremental alcohol dehydration and then embedded in PMMA (Technovit 7200, Exakt Technologies, Oklahoma City, OK, USA). After preparation of the tissue blocks, a plastic slide was bonded to the block and slides were cut in 200 µm thick sections, using a diamond-blade saw, and then polished to a final thickness between 50 and 90 µm (Exakt Cutting and Grinding System, Exakt Technologies, Hamburg, Germany). Slide orientataion for cutting was done on the longitudinal axis of the pin in a frontal plane to the bone. One slide was made at a similar location for each of the designated specimens. Slides were stained with toluidine blue at a pH = 9.0 for histomorphometric analysis.

Histomorphometric analysis included determination of an affinity index (AI), osteoclast index (OI), interface histological score (IHS) and % trabecular bone area (%TBA). An image analysis system (Bioquant-OS/2, Rand M Biometrics, Nashville, TN, USA) was used to measure the length of cortical bone bound to the SPCP or in contact with the implant. The ratio of the length of direct bone contact to the length of implant traversing each cortex was the AI for each specimen. The OI was determined by counting the number of osteoclasts per high power field (hpf). 5 fields were counted per specimen and the average number for each specimen was recorded as the OI. The interface histological score (adapted from Jansen et al. 1993) was defined as follows: 4) direct cortical bone contact with the implant surface, 3) irregular or remodeling bone adjacent to the implant, 2) intermittent fibrous tissue and bone in contact with the implant, 1) fibrous tissue surrounding the implant and 0) inflammation surrounding the implant. The IHS was determined for each cortex and averaged to determine the IHS for each specimen. An image analysis system (Bioscan Optimus, Bioscan Incorporated, Edmonds, WA, USA) was used to determine the %TBA by measuring the surface area of bone within the defined region surrounding the portion of the implant in the medullary canal. Data for AI, OI, IHS, and %TBA were averaged for each calf and the mean was used for statistical comparisons.

A chi-square test with Yates' correction was used for categorical data (pin site discharge, osteolysis data). Fisher's exact test was used when the count in one category or more was less than 5. A two-factor (treatment, time) analysis of variance was used for data from nuclear scintimetry. The t-test was used to analyze data from mechanical tests, affinity index, osteoclast index, % trabecular bone volume and interface histology score. In all statistical tests, a p-value $\leq$ 0.05 was considered significant.

## Results (Table)

Discharge was observed from fewer pins (5/24) in the SPCP group calves than in control group calves (21/24) during the study ($p = 0.01$). Discharge varied in character from serous to fibrinous and had no odor, except in 2 calves in the control group. The SPCP group of calves had significantly fewer calves (2/6) with discharging pins compared with the control group calves (6/6; $p = 0.03$).

Radiographic images obtained immediately after surgery and at the end of the study confirmed proper pin placement in all calves. Pin bending, fracture fragment displacement or cortical bone fracture adjacent to threaded pins were not observed during the study period. Osteolysis was seen in more (10/24)

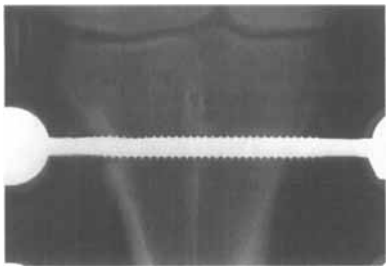

Figure 2. Dorsopalmar projected radiographic image of the proximal metaphyseal pin in a control group calf's metacarpus 40 days after surgery. Note radiographic rarefaction indicating pin tract osteolysis of exit cortex.

**Osseointeraction of solution-precipitated calcium phosphate (SPCP)-coated and uncoated (Control) threaded transfixation pins used in external skeletal fixation of a calf stable fracture model , mean (SD)**

| | SPCP | Control | P-value |
|---|---|---|---|
| $^{99m}$Tc-MDP gamma count ratio | | | |
| 6 days | 1.74 (0.40) | 1.33 (0.58) | |
| 28 days | 1.51 (0.35) | 1.74 (0.22) | 0.6 |
| Yield stress (Mpa) | 9.3 (1.0) | 8.5 (1.4) | 0.1 |
| Peak force (kN) | 3.7 (0.4) | 3.4 (0.6) | 0.2 |
| BMD (g/cm$^2$) | 0.71 (0.05) | 0.77 (0.07) | 0.05 |
| kN/BMD [a] | 5.2 (0.4) | 4.4 (0.9) | 0.04 |
| Affinity index | 0.56 (0.13) | 0.34 (0.13) | 0.01 |
| OI (osteoclasts/hpf) | 0.88 (0.44) | 2.67 (2.4) | 0.05 |
| IHS [b] | 2.93 (0.34) | 1.96 (0.71) | 0.01 |
| %TBA [c] | 14 (2) | 14 (6) | 0.4 |

[a] Resistance to axial displacement/unit bone mineral density.
[b] Interface histological score.
[c] Percentage trabecular bone area

Figure 3. Bone coating-pin interface histologic image. Note bone bonding onto the solution-precipitated calcium phosphate-coated transfixation pin. Magnification 40 ×, toluidine blue stain (pH = 9.0).

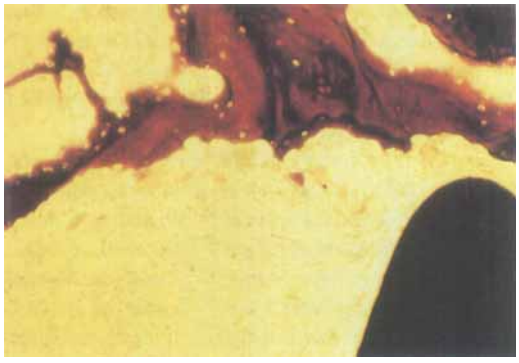

Figure 4. Histologic image of bone-pin interface of a transfixation pin (without coating). Note resorption lacuna on the bone surfaces adjacent to pin. Magnification 25 ×, toluidine blue stain (pH = 9.0).

pins in the control group than in the SPCP group (1/24) pins (p = 0.01; Figure 2). However, the number of SPCP group calves with osteolysis (1/6 calves) was not different compared to control group calves (4/6 calves; p = 0.1).

Uptake of $^{99m}$Tc-MDP was similar for the treated limb 6 days after surgery (Table) compared to 28 days after surgery for both treatment groups (p = 0.6). Radioisotope uptake was also similar in the SPCP group calves and control calves 6 days and 28 days after surgery (p = 0.6). Radioisotope uptake was greater in bone segments with regions of radiographic evidence of osteolysis than in bone segments not affected by osteolysis (p = 0.03).

The yield stress and peak force for axial displacement of pins in the SPCP group were higher than for control group pins, but the difference was not significant (p = 0.1, 0.2, respectively). Bone mineral density (BMD) was less for bone surrounding pins in SPCP group calves compared with control group calves (p = 0.05). Analysis of resistance to axial displacement/unit bone mineral density was greater for the SPCP group compared with the control group (p = 0.04).

Mean AI was higher for SPCP group pins compared to control group pins (p = 0.01). Bone bonding to the SPCP coating was seen (Figure 3). The SPCP coating appeared to have survived the initial insertion torque without obvious disruption. Mean OI was less in SPCP group pins than for the control group pins (p = 0.05). Fibroplasia and resorption lacuna were seen surrounding many control pins (Figure 4). Mean IHS was higher for SPCP group calves, compared to control group calves (p = 0.01). Mean %TBA for SPCP group calves was similar to that for control group calves (p = 0.4).

## Discussion

We hypothesized that solution-precipitated calcium phosphate-coated transcortical pins used in external

fixation would result in rapid bone bonding and improved osseointeraction of implants. Our findings support this hypothesis. Calcium phosphate coating of transfixation pins effectively reduced the development of pin discharge and radiographic evidence of osteolysis. Immediately after implantation, a chemical bond forms between the calcium phosphate coating and the bone. This effect has been observed within 2 to 3 weeks after implantation (Ducheyne et al. 1990, Burr et al. 1993). Non-coated implants were surrounded by a fibrous membrane. We hypothesized that rapid bone bonding of SPCP-coated transfixation pins would reduce the development of osteolysis and pin loosening. Premature pin loosening was not recognized in this study. However, discharge and osteolysis were significantly reduced in pins coated with solution-precipitated calcium phosphate.

No adverse tissue reactions caused by the presence of the SPCP coating were observed during this study. Uptake of $^{99m}$Tc-MDP was similar in the two treatment groups, implying that no adverse bone reaction to the coating had occurred. Increased uptake of the $^{99m}$Tc-MDP in some control calves 28 days after surgery was caused by the development of osteolysis with concurrent bone remodeling around the pins.

Histomorphometric analysis of the cortical bone-implant interface demonstrated improved osseointeraction of the SPCP-coated pins. Direct bone-implant contact, as determined by affinity index (Hayashi et al. 1989), was greater for SPCP pins. Bone reaction around control pins was characterized by a higher osteoclastic index (Branemark 1983) and more fibrous tissue formation than in SPCP-coated pins. The similar % trabecular bone area for SPCP-coated and non-coated pins in this study was in contrast to studies involving hydroxyapatite ceramic-coated porous implants inserted in regions of trabecular bone (Ducheyne et al. 1990, Soballe et al. 1992). Trabecular bone deposition around pins in the control group may have been secondary to pin tract osteolysis and subsequent osseous modeling around the pin in the medullary region.

Dual-energy x-ray absorptiometry (DEXA) is a precise, accurate, nondestructive method of estimating bone mineral density with a small coefficient of variation (Kelly et al. 1988). Storage of bone segments in 10% neutral buffered formalin has been found not to change the results of DEXA analysis after 60 days of storage (Toll et al. 1994). Bone mineral density (BMD) has been shown to be highly, positively correlated to mechanical tests involving cadaver specimens (Alho et al. 1988). Although DEXA analysis of BMD is precise, correlation of mechanical response of in vivo implant models to BMD may not be useful, because of ultrastructural changes at the level of the bone-implant interface. Osseous remodeling around the implant, secondary to pin tract osteolysis and bone remodeling, may result in a higher BMD which does not contribute to the mechanical strength of the bone-implant interface. In our study, the control group had higher BMD but lower yield stress. DEXA analysis may be better for serial evaluation of bone-implant reactions.

Resistance to axial displacement of SPCP transfixation pins averaged 15% higher than those of the control group. Use of positive profile threaded transfixation pins was expected to limit differences between the two groups. Tests of removal torque are more precise measurements of the bone-implant interface. However, we used push-out tests because transfixation pin morbidity is usually associated with axial displacement (shear force). Therefore, we wanted to determine whether SPCP would increase the resistance to axial displacement of positive profile pins. These tests more accurately reflect conditions contributing to transfixation pin failure under clinical conditions. We could speculate that significant differences would have been noted after a longer study period (e.g., 90 days).

Recently, a study using hydroxyapatite-coated (plasma spray) threaded half-pins for monoaxial fixation of a tibial osteotomy in sheep has been published (Stea et al. 1995). Although statistical differences were not found between treatment groups, this study indicated that hydroxyapatite-coated pins had a tendency for greater bone contact and bone ingrowth compared with control pins. In our study, coating of transfixation pins used in external skeletal fixation with solution-precipitated calcium phosphate resulted in improved bone-implant osseointeraction compared with control pins.

The animal model used for our study differs from that previously reported for hydroxyapatite ceramic research. Calves tolerate orthopedic procedures well, allowing immediate postoperative quadrupedal ambulation. The long bones of calves are composed of plexiform or laminar bone and bone-implant interactions are characterized by modeling of bone. This allowed evaluation of bone modeling around the implants, instead of evaluation of bone remodeling. We feel that this provides important information regarding bone bonding and biocompatability. Laminar bone specimens may survive nondestructive mechanical tests (yield stress) better than lamellar bone specimens, allowing subsequent histomorphometric analyses to be performed.

Solution-precipitated calcium phosphate coating of transcortical implants may represent an important ad-

junct in external skeletal fixation technology. To date, pin tract osteolysis, premature pin loosening and pin tract infection remain the major limitations in the application of external skeletal fixation. Improved osseointeraction by solution-precipitated calcium phosphate coating of transcortical implants may increase the longevity and reduce the morbidity from complications associated with the use of transfixation pins.

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