Effects of orthopedic implants with a polycaprolactone polymer coating containing bone morphogenetic protein-2 on osseointegration in bones of sheep

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Objective—To determine elution characteristics of bone morphogenetic protein (BMP)-2 from a polycaprolactone coating applied to orthopedic implants and determine effects of this coating on osseointegration.

Animals—6 sheep.

Procedures—An in vitro study was conducted to determine BMP-2 elution from polycaprolactone-coated implants. An in vivo study was conducted to determine the effects on osseointegration when the polycaprolactone with BMP-2 coating was applied to bone screws. Osseointegration was assessed via radiography, measurement of peak removal torque and bone mineral density, and histomorphometric analysis. Physiologic response was assessed by measuring serum bone-specific alkaline phosphatase activity and uptake of bone markers.

Results—Mean $\pm$ SD elution on day 1 of the in vitro study was 263 $\pm$ 152 pg/d, which then maintained a plateau at 59.8 $\pm$ 29.1 pg/d. Mean peak removal torque for screws coated with polycalprolactone and BMP-2 (0.91 $\pm$ 0.65 dN•m) and screws coated with polycaprolactone alone (0.97 $\pm$ 1.30 dN•m) did not differ significantly from that for the control screws (2.34 $\pm$ 1.62 dN•m). Mean bone mineral densities were 0.535 $\pm$ 0.060 g/cm^2, 0.596 $\pm$ 0.093 g/cm^2, and 0.524 $\pm$ 0.142 g/cm^2 for the polycaprolactone–BMP-2–coated, polycaprolactone-coated, and control screws, respectively, and did not differ significantly among groups. Histologically, bone was in closer apposition to the implant with the control screws than with either of the coated screws.

Conclusions and Clinical Relevance—BMP-2 within the polycaprolactone coating did not stimulate osteogenesis. The polycaprolactone coating appeared to cause a barrier effect that prevented formation of new bone. A longer period or use of another carrier polymer may result in increased osseointegration. (*Am J Vet Res* 2009;70:1416–1425)

The bone-implant interface is frequently the weak link in the viability of orthopedic constructs, and suboptimal bone-implant interface reactions are the most common cause for implant failure or complications resulting in premature removal of implants.[1] Aseptic loosening of orthopedic implants is the most common cause of revision of major arthroplasties in humans.[2] Loosening of a prosthetic is the most common complication of total knee arthroplasties in humans, with an incidence of approximately 10%.[3,4] Aseptic loosening of the femoral component is reportedly the

second most common complication reported in canine total hip arthroplasty.[5] It has been determined that 20%[6] to 41%[7] of cortically placed screws in dogs undergoing triple pelvic osteotomy had screw loosening. Of 16 dogs undergoing triple pelvic osteotomy, 9 had loose screws by 10 days after implantation.[6]

Osseointegration refers to the growth of bone as it incorporates surgically implanted materials. Ideally, osseointegration continues until an intimate apposition of bone and biomaterial is achieved. The process of osseointegration involves the behavior of the material in the host and the response of the host to the implant. Extrinsic factors such as premature loading of the bone-implant construct can affect osseointegration.[1]

Bone morphogenetic proteins are growth factors that belong to the transforming growth factor β superfamily. There are many classes of BMPs, but of these, BMP-2 is reported to be the most potent osteoinductive factor.[8] Bone morphogenetic protein-2 has been used

ABBREVIATIONS

BALP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
BMP	Bone morphogenetic protein

most often in an attempt to affect the osseointegration of orthopedic implants, and there are abundant data to support its use.[9–17] Most studies[13,18,19] have involved implantation in the medullary cavity or facial bones, but little research has been done to evaluate application of BMP-2 to the cortical interface of long bones.

The objective of the study reported here was to determine whether a BMP-2 coating would increase the osseointegration of a metal implant in sheep. We hypothesized that polycaprolactone, a biodegradable polymer, would serve as a scaffolding to bind to the screws, allow for impregnation of BMP-2, and release BMP-2 over time, which would allow the BMP-2 to have a biological effect. We further hypothesized that the coating of orthopedic implants with BMP-2 would stimulate more rapid osseointegration of bone around transcortical implants with intracortical gaps, such as is evident during placement of screws in lag fashion.

Materials and Methods

Implant preparation—For the in vitro and in vivo experiments conducted during this study, 3.5-mm-diameter, 316L, stainless-steel cortical bone screws were used. Poly(ε-caprolactone)[a] with a molecular weight of 65,000 Da was heated to 120°C in a 500-mL glass beaker. Bone screws were dipped into this molten polymer to completely cover the threads. Bone screws were then suspended from a metal screen in a vacuum oven heated to 120°C and held overnight to encourage excess polymer to passively drain from the threads. After completion of this process, a No. 15 scalpel blade was used to carefully remove a small section of the coating from the surface of the threads. Thickness of the section of coating was measured with digital calipers[b] and was determined to be approximately 25 μm. An osmium coater[c] was used to deposit a 15-nm-thick film of osmium metal onto that portion of the thread from which the aforementioned section of polymer coating was removed. Screws were then evaluated by use of a scanning electron microscope[d] at 5 kV to confirm that the thickness of the remaining polycaprolactone coating was approximately 25 μm.

The polycaprolactone-coated screws were then submerged in a solution of human-recombinant BMP-2[e] that contained 10 μg of BMP-2/mL of deionized water. Both the screws and solution were then exposed to bone-dry–grade carbon dioxide (99.99% pure)[f] at 3.4 MPa for 2 hours at 20°C by use of a high-pressure syringe pump.[g] In other studies[20–22] conducted by our laboratory group, it has been determined that these exposures allow for infusion of compounds into polymers in general and polycaprolactone in particular. The pressure was then slowly decreased during a 6.5-hour period to ensure that no bubbles of carbon dioxide formed and that the polycaprolactone coating remained intact.

In vitro evaluation of BMP-2 elution from polycaprolactone–BMP-2 coating on bone screws—Four 3.5-mm-diameter, 316L, stainless-steel cortical bone screws (30 mm in length) were used for in vitro testing. These polycaprolactone–BMP-2–coated screws were completely submerged in 2 mL of PBS solution (137mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, and 1.8mM KH_2PO_4 [pH, 7.4]) in individual sterile glass tubes capped with rubber stoppers and incubated at 37°C (day 0). Supernatant was removed for subsequent BMP-2 assay on days 1, 4, 7, 10, 14, 17, 21, 25, and 31. Each screw was then rinsed with 3 mL of fresh PBS solution and completely submerged in 2 mL of fresh PBS solution in a new sterile glass tube capped with a rubber stopper. The supernatant was placed in cryotubes and stored at –80°C until BMP-2 assays were performed. Concentration of BMP-2 in the PBS solution was measured with a commercial BMP-2 ELISA kit.[h]

In vivo evaluation of polycaprolactone–BMP-2 coating on bone screws—The experimental protocol for the in vivo portion of this study was approved by The Ohio State University Laboratory Animal Care and Use Committee.

ANIMALS

Six healthy mature sheep (5 Suffolk and 1 Dorset; 2 rams and 4 ewes) were used for the experiment. The rams were yearlings, and the ewes ranged from 3 to 5 years of age. All sheep were housed in box stalls (4×4-m) throughout the study period. Sheep were provided hay and water ad libitum. All food was withheld for 12 hours prior to surgery. Beginning immediately before surgery, administration of flunixin meglumine (1 mg/kg, IV, q 24 h for 3 days) and ceftiofur hydrochloride (2 mg/kg, SC, q 24 h for 7 days) was initiated. The day of surgery was designated as day 0.

Physical examinations were performed on all 6 sheep. Rectal temperature, heart rate, respiratory rate, and lameness were assessed on a daily basis throughout the experiment. Limb pain was subjectively assessed daily on a scale of 0 to 5 (0 = free of pain; 1 = walks freely with limp; 2 = walks reluctantly with limp; 3 = increased recumbency, reluctant to walk; 4 = walks but will not bear weight on limb; and 5 = recumbent, will not stand). Body weights were obtained on a weekly basis throughout the experiment.

ANESTHESIA

An indwelling 14-gauge, 12.5-cm-long catheter was placed aseptically in a jugular vein of each sheep. Anesthesia was induced with a solution containing 500 mg of ketamine hydrochloride in 500 mL of 5% guaifenesin (2.2 mL/kg, IV). Endotracheal intubation was performed, and anesthesia was maintained with isoflurane vaporized in 100% oxygen.

SURGICAL PROCEDURES

After induction of anesthesia, sheep were positioned in dorsal recumbency, and the limbs were suspended. A No. 40 surgical clipper blade was used to clip the wool from the right forelimb and right hind limb. The right metacarpus and right metatarsus were aseptically prepared with povidone iodine surgical scrub and rinsed with 70% isopropyl alcohol.

Cortical bone screws (22-mm-long, 3.5-mm-diameter, 316L, stainless-steel screws) were used. Bone screws comprised 3 groups: control screws (n = 18) for standard placement in a lag manner, polycaprolactone-coated screws (9) for evaluation of the effect of poly-

mer coating on the bone-implant interface, and poly-caprolactone–BMP-2–coated screws (9) for evaluation of the effect of BMP-2–impregnated polymer coating on the bone-implant interface. All screws were disinfected within 5 minutes prior to implantation by submerging them in 95% ethyl alcohol for 1 minute. Each screw was rinsed with sterile saline (0.9% NaCl) solution prior to implantation.

A 6-cm linear incision was made on the lateral aspect of the right forelimb and hind limb of each sheep over the diaphysis of the metatarsus or metacarpus. The incision penetrated the skin, subcutaneous tissues, and periosteum. Soft tissues were retracted, and a 2.7-mm drill bit was used to drill through both the cis and trans cortices of the diaphysis of the metacarpus or metatarsus in a lateral to medial direction. A 3.5-mm tap was used to tap each hole after drilling, and the cis cortex was overdrilled with a 3.5-mm drill bit. A 22-mm-long, 3.5-mm-diameter bone screw was placed in the defect. Only the trans cortex was engaged by the bone screw. The cis cortex was used as a gap defect model to simulate lag screw implants. Three bone screws (proximal, middle, and distal; spaced approximately 2 cm apart) were implanted in each limb. Each metacarpus and metatarsus was randomly assigned to receive polycaprolactone–BMP-2–coated, polycaprolactone-coated, or uncoated (control) screws (**Appendix**). Each sheep had 1 limb assigned to the control screws and 1 limb assigned to the polycaprolactone-coated or the polycaprolactone–BMP-2–coated group. A Latin square design was used to ensure balance among groups.

After implantation of screws, skin incisions were closed with 1-polyglactin 910[i] in a Ford interlocking pattern. The right metacarpus and metatarsus of each sheep were bandaged, and the sheep were allowed to recover from anesthesia. Following recovery, hay and water were offered ad libitum.

Radiographic evaluation

Radiographic images of the limbs were obtained with a digital radiography unit.[j,k] Radiographs were obtained weekly beginning the day after surgery. Two orthogonal radiographic views (lateral and dorsopalmar-dorsoplantar) of each right metatarsus and right metacarpus were obtained.

Digital radiographs were viewed via a computer software program.[l] All radiographs were assessed by a board-certified veterinary radiologist (VFS) who had no knowledge of the treatment groups. The radiographic response was assessed for the area around the cis cortex of the screw in 4 regions, the periosteum, the cortical bone, the endosteum, and the medullary cavity. A subjective scoring system (–1 = decrease in radiographic opacity; 0 = no change in radiographic opacity; and 1 = increase in radiographic opacity) was used for radiographic assessment. At day 0, all scores were assigned a value of 0, and all subsequent images were compared to the day 0 image. When opacity was detected in the medullary cavity, it was considered an artifact of surgical implantation (debris from drilling). This opacity was taken into account when evaluating subsequent radiographs. A composite score was calculated for each anatomic region on each limb for each day. The composite score was calculated by calculating the mean of all 3 scores for that region on each day.

Uptake of bone markers

Oxytetracycline[m] was administered (20 mg/kg, IV) immediately prior to surgery to each sheep for use as a bone marker. On day 14 after surgery, a green fluorescent dye[n] was administered (20 mg/kg, IV) to each sheep. The green fluorescent dye was dissolved in 2% sodium bicarbonate solution. On day 28 after surgery, oxytetracycline (20 mg/kg, IV) was again administered to each sheep.

Harvest of bone screws

Thirty-six days after surgery, each sheep was euthanatized and the right forelimb and hind limb were harvested. Cross sections of each right metacarpus and right metatarsus were created so that each bone was divided into 3 sections (ie, samples), with each sample containing 1 screw. The samples were submitted for histomorphologic analysis of the bone adjacent to the screws or for measurement of peak removal torque of the screws (Appendix). Samples submitted for histomorphologic analysis were then subsequently analyzed for BMD. Identical analyses were performed on corresponding samples from ipsilateral limbs (forelimb vs hind limb) so that a control sample was matched with each experimental sample from the same sheep.

Peak removal torque

All samples were harvested immediately after sheep were euthanatized. Samples were stored at –80°C until analysis was performed. All samples were thawed at 20°C for 24 hours prior to testing.

Peak removal torque was measured for 16 screws (4 polycaprolactone-coated screws, 4 polycaprolactone–BMP-2–coated screws, and 8 control screws). Removal torque was determined by use of a torque meter.[o] The heads of all screws were dissected free of soft tissue. A commercial hexagonal, 3.5-mm, orthopedic screwdriver head[p] was modified to fit onto the end of the torque meter load cell.[q] Each limb was held stationary against the table by an assistant while each screw was turned in a counterclockwise direction 1 full turn and peak removal toque was recorded. Effort was made to turn each of the screws at a constant rate. A 1-way ANOVA was performed to determine whether a significant difference existed among groups. A Kruskal-Wallis test was also used to compare groups in the case that the assumption of normality was not valid. Significance was set at values of $P < 0.05$.

Histomorphologic analysis and osteonal activity

Specimens consisted of a single screw implanted in a section (approx 3 or 4 cm) of diaphyseal bone. Twenty specimens were analyzed, consisting of 5 polycaprolactone-coated screws, 5 polycaprolactone–BMP-2–coated screws, and 10 control screws. Specimens were stored and shipped in 70% ethanol to prevent decalcification. Specimens were sectioned with a commercial cutting and grinding system[r] for histomorphologic analysis by cross section of the bone along the longitudinal axis of the screw.

Two slides were created for each specimen. One slide was stained with toluidine blue, and the other was left unstained for UV examination of bone markers.

Histologic sections were analyzed via light microscopy at 100× magnification. The sections were obtained in a transverse plane, parallel to the implant and perpendicular to the long axis of the bone, and traveling through the center of the implant. The cis cortex gap region was analyzed histologically (**Figure 1**). Each thread was assigned an identification number. The area between the threads of each screw at the cortical bone-screw interface at the cis cortex was analyzed and subjectively assessed (bone resorption, no change, or new bone formation).

Unstained preparations were viewed under UV light at 400 nm to detect bone markers to assess osteonal activity. Oxytetracycline fluoresced as a yellow-orange band, and the green fluorescent dye fluoresced as a bright green band. Preparations were viewed by use of a microscope[s] with a fluorescein di-isothiocyanate filter (200× magnification) attached to a UV light burner.[t] In each preparation, 100 osteons were counted, and the number of osteons with uptake of oxytetracycline and green fluorescent dye was recorded. Because oxytetracycline was administered to the sheep twice during the study, the time of oxytetracycline uptake could only be determined if there was uptake of another bone marker. When 2 markers were evident, the spatial relationship of the markers could be used to determine the dates of marker uptake. The fraction of osteonal activation at the beginning, middle, and end of the study (days 0, 14, and 28, respectively) was determined.

EVALUATION OF BMD

Dual energy x-ray absorbtometry[u] was used to quantitatively measure BMD around the screws. Scans were performed at 140 and 70 kVP and a mean of 2.0 mA. Scans were performed in planes parallel to the long axis of the screw. The scanner was set for lumbar vertebral scanning with a single beam. Line spacing and point resolution was set at 0.10 cm.

Following each scan, analysis was performed by dividing the scanned area into 2 regions of interest.
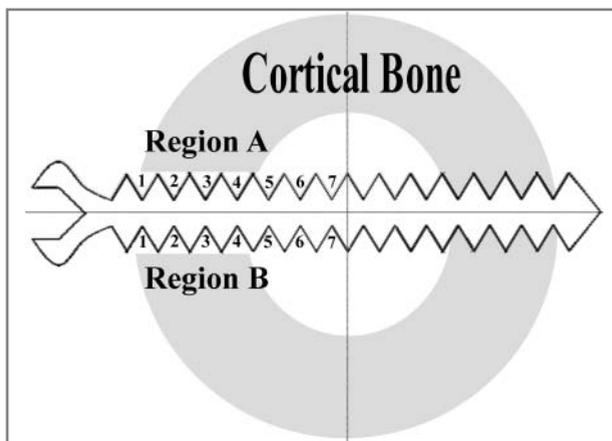

Figure 1—Regions of the histomorphometric section of a bone into which a cortical bone screw was implanted. Each section was divided into 2 regions (A and B, respectively). Each region contained approximately 7 gaps between threads, although the actual number of gaps varied.

Region 1 included the bone parallel and immediately adjacent to one side of the screw, and region 2 included the bone parallel and immediately adjacent to the other side of the screw (**Figure 2**). After the regions were labeled and outlined, the system processed the scanned image to create a bone map. The area and BMD for each region were calculated, and a mean BMD value for regions 1 and 2 was calculated.

A 1-way ANOVA was used to assess differences among the 3 groups. In addition to the 1-way ANOVA, a Kruskal-Wallis test was used to analyze the data in the case that the assumption of normality was not valid. Analysis was performed among the treatment groups and their ipsilateral control limbs by use of a paired t test. Significance was set at values of $P < 0.05$.

EVALUATION OF BALP ACTIVITY

A blood sample was collected from each sheep on days 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 after surgery. Blood was allowed to clot, and the samples were then centrifuged. Serum was harvested, placed in cryotubes, frozen, and stored at
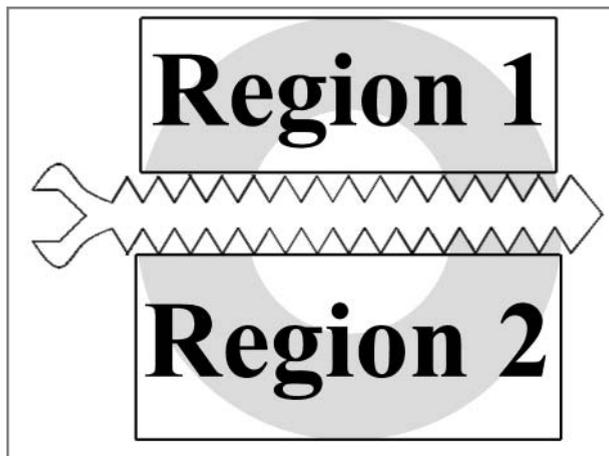

Figure 2—Regions of the sections of a bone defined for purposes of dual-energy x-ray absorptiometry. Region 1 was defined as the area of cortical bone immediately adjacent to one side of the screw, and region 2 was defined as the area of cortical bone immediately adjacent to the other side of the screw.
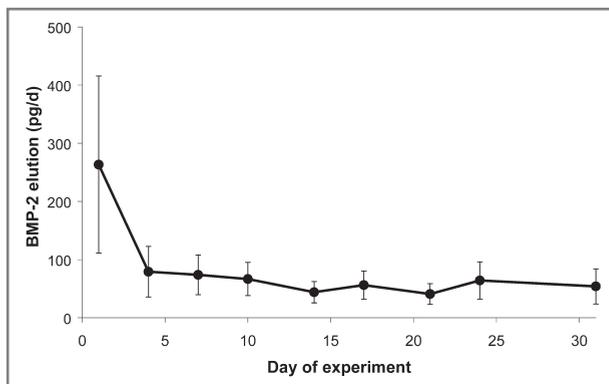

Figure 3—Mean ± SD BMP-2 elution from polycaprolactone–BMP-2–coated screws in an in vitro experiment. The highest BMP-2 elution was at the beginning of the study (day 0 = day of insertion of implants into tubes containing PBS solution). Notice that the BMP-2 elution remained relatively constant throughout the remainder of the experiment.

Table 1—Radiographic scores for the cis cortex adjacent to polycaprolactone-coated, polycaprolactone–BMP-2–coated, or uncoated (control) screws (3 samples/limb; a proximal, middle, and distal screw) implanted in the right metacarpus (MC) and right metatarsus (MT) of each of 6 sheep.

Sheep No.	Screw location	Day 0		Day 7		Day 14		Day 22	
		MC	MT	MC	MT	MC	MT	MC	MT
1	Proximal	0	0*	0	0*	1	1*	1	1*
	Middle	0	0*	0	0*	1	1*	1	1*
	Distal	0	0*	0	0*	0	1*	1	1*
2	Proximal	0†	0	0†	0	0†	0	1†	1
	Middle	0†	0	0†	0	1†	0	1†	1
	Distal	0†	0	0†	0	0†	0	1†	1
3	Proximal	0	0*	0	0*	1	0*	1	1*
	Middle	0	0*	0	0*	0	0*	0	0*
	Distal	0	0*	0	0*	0	0*	0	0*
4	Proximal	0	0†	0	0†	0	0†	1	−1†
	Middle	0	0†	0	0†	0	−1†	1	−1†
	Distal	0	0†	0	0†	0	0†	1	−1†
5	Proximal	0	0†	1	0†	1	0†	1	0†
	Middle	0	0†	1	0†	1	0†	1	0†
	Distal	0	0†	0	0†	0	0†	0	0†
6	Proximal	0*	0	0*	0	0*	0	0*	0
	Middle	0*	0	0*	0	0*	0	0*	1
	Distal	0*	0	0*	0	0*	0	0*	0

All scores reflect the radiographic opacity, compared with the opacity on the day of surgery (day 0). Values were assigned on the basis of the following scale: –1 = decrease in radiographic opacity; 0 = no change in radiographic opacity; and 1 = increase in radiographic opacity. Identical analyses were performed on corresponding samples from ipsilateral limbs of each sheep so that a control sample was matched with each experimental sample.
*Polycaprolactone-coated screw. †Polycaprolactone–BMP-2–coated screw.

–80°C until analysis was performed to measure activity of BALP. The BALP activity was assayed via a wheat-germ lectin precipitation method.[v] An unpaired t test was used to compare the mean serum BALP activity of sheep in the polycaprolactone–BMP-2 group with mean serum BALP activity of sheep in the polycaprolactone group. When the assumption of normality was false, a Mann-Whitney U test was used to assess differences among the groups. Values of $P < 0.05$ were considered significant.

Results

Elution of BMP-2—Elution of BMP-2 was detected throughout the 31 days of the in vitro study period. Mean ± SD elution for the first day of the study was 263 ± 152 pg/d. After day 1, BMP elution remained relatively constant throughout the remainder of the study and varied from 79.2 ± 43.6 pg/d to 40.9 ± 17.7 pg/d (**Figure 3**).

In vivo experiment—Results were determined for the various components of the in vivo portion of the study.

ANIMALS
Mean ± SD body weight of the sheep was 98 ± 16 kg at day 0 and 96 ± 14 kg at day 30. Body condition scores ranged from 3 to 4 (scale of 1 to 5) during the study. No complications were detected during anesthesia or surgery. None of the sheep were lame following the procedure (lameness score = 0 for all sheep), except for 2 sheep (1 sheep had a grade 1 lameness of the right forelimb on day 24 that resolved without treatment by the end of day 25, and the second sheep had a grade 1 lameness on the last day of the study period).

Table 2—Mean ± SD percentage of osteons with uptake of bone marker in the right metacarpus and right metatarsus of 6 sheep at day 0, day 14, and day 28 of the experiment.

Group	Day 0	Day 14	Day 28
Control	3.7 ± 8.2	10.9 ± 17.0	1.0 ± 2.8
PCL	6.0 ± 12.3	10.2 ± 21.7	0 ± 0
PCL–BMP-2	3.4 ± 4.7	14.6 ± 15.3	0 ± 0

PCL = Polycaprolactone-coated screws. PCL–BMP-2 = Polycaprolactone–BMP-2–coated screws.

RADIOGRAPHIC EVALUATION
Scores for the radiographic assessments of the cis cortex in the areas adjacent to the screws for the right metacarpus and metatarsus were summarized (**Table 1**). Most screws had an increase in radiographic opacity adjacent to the screw of the cis cortex by day 22. The only screws that had a decrease in radiographic opacity during the course of the study were the metatarsal screws of a sheep in the polycaprolactone–BMP-2 group. The middle screw had a decrease in radiographic opacity by day 14, and all 3 screws in this limb had a decrease in radiographic opacity by day 22.

UPTAKE OF BONE MARKERS
More osteons had uptake of bone marker on day 14 than at any other time during the study. The percentage of osteons that had uptake of bone marker at days 0, 14, and 28 of the study was determined (**Table 2**).

PEAK REMOVAL TORQUE
Sixteen screws were evaluated for peak removal torque. Mean ± SD peak removal torque for the polycaprolactone–BMP-2–coated screws was 0.91 ± 0.65 dN•m
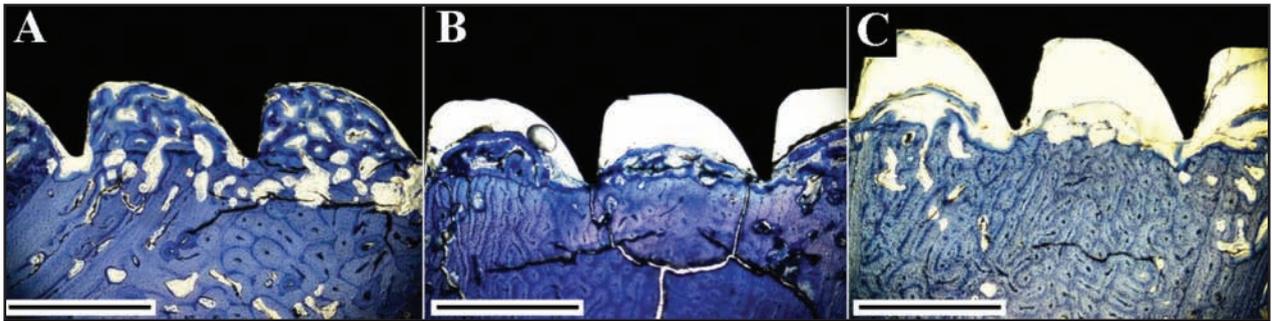
Figure 4—Photomicrographs of tissue sections obtained from the bone-implant interface of an uncoated screw (A), a polycaprolactone-coated screw (B), and a polycaprolactone–BMP-2–coated screw (C). In panels B and C, notice that there is little to no new bone formation between the threads of the coated screws and that there is white space between the screw and bone in the polycaprolactone-coated and polycaprolactone–BMP-2–coated screws. It is speculated that the polymer coating was removed during processing. Toluidine blue stain; bar = 1 mm.

Table 3—Results for histomorphologic analysis of gaps between threads of the cis cortex in bone samples obtained from 6 sheep.

Group	Bone resorption	No change	New bone growth	Total No. of gaps counted
PCL	14	14	0	28
PCL–BMP-2	5	15	9	29
Control	0	2	72	74

Scores were assigned by use of a subjective scoring system based on no change, new bone growth, and bone resorption.
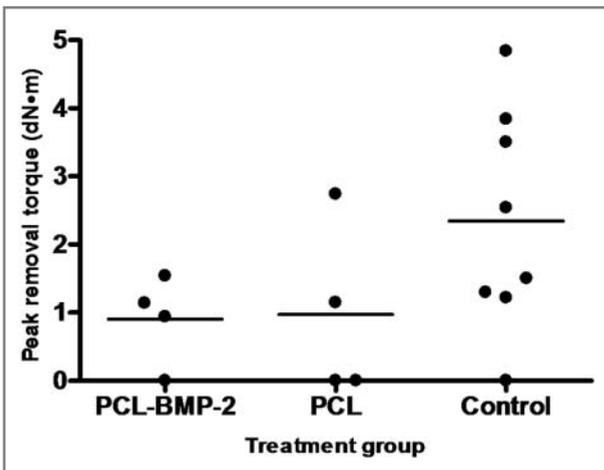See Table 2 for remainder of key.

Figure 5—Peak removal torque for extraction of screws from bones of sheep. Each symbol represents results for 1 screw. Horizontal bars represent the mean of the peak removal torques. Mean peak removal torques for the coated (polycaprolactone [PCL] and PCL–BMP-2) screws were lower than, but did not differ significantly ($P \geq 0.05$) from, the mean for the control screws.

(range, 0 to 1.54 dN•m). Mean peak removal torque for polycaprolactone-coated and control screws was 0.97 ± 1.30 dN•m (range, 0 to 2.74 dN•m) and 2.34 ± 1.62 dN•m (range, 0 to 4.84 dN•m), respectively (Figure 4). These values did not differ significantly ($P = 0.237$ [1-way ANOVA] and $P = 0.257$ [Kruskal-Wallis test]).

HISTOMORPHOLOGIC ANALYSIS

Twenty specimens were analyzed. The number of threads contained within the cortical bone region

varied from 2 to 5 threads. For the 10 control screws, there was new bone growth between 72 of 74 threads evaluated. Only 9 of 29 threads of the 5 polycaprolactone–BMP-2–coated screws had evidence of new bone formation, and there were no threads of the 5 polycaprolactone-coated screws that had new bone formation (Table 3).

Representative histologic sections for the 3 groups at the implant cortical margin and the implant periosteal margin were evaluated (Figure 5). New bone was visible within the area between the threads of the control screws, whereas little to no new bone was visible between the threads of the coated screws (polycaprolactone-coated and polycaprolactone–BMP-2–coated screws).

EVALUATION OF BMD

Area of the bone map ranged from 0.74 to 2.82 cm² (mean ± SD, 1.51 ± 0.52 cm²). The BMD ranged from 0.160 to 0.707 g/cm² (mean, 0.545 ± 0.114 g/cm²). The mean BMD was 0.524 ± 0.142 g/cm², 0.596 ± 0.093 g/cm², and 0.535 ± 0.060 g/cm² for the control, polycaprolactone-coated, and polycaprolactone–BMP-2–coated screws, respectively. The BMD did not differ significantly ($P = 0.5225$ [1-way ANOVA] and $P = 0.5128$ [Kruskal-Wallis test]) among the groups.

BALP ACTIVITY

The serum BALP activity remained relatively constant throughout the study for all sheep, except for 1 sheep in the polycaprolactone-coated group and 1 sheep in the polycaprolactone–BMP-2–coated group, both of which had a spike in BALP activity during the first 3 days of the study (Figure 6). Serum BALP activity did not differ significantly ($P = 0.931$ [unpaired t test] and $P = 1.000$ [Mann-Whitney U test]) when values for sheep from the polycaprolactone-coated and polycaprolactone–BMP-2–coated groups were compared.

Discussion

Polycaprolactone is a potential drug-delivery system for BMP-2 as determined on the basis of the in vitro elution data for the study reported here. Elution was characterized by a burst release of BMP-2 during the first 24 hours, followed by a plateau in the release for
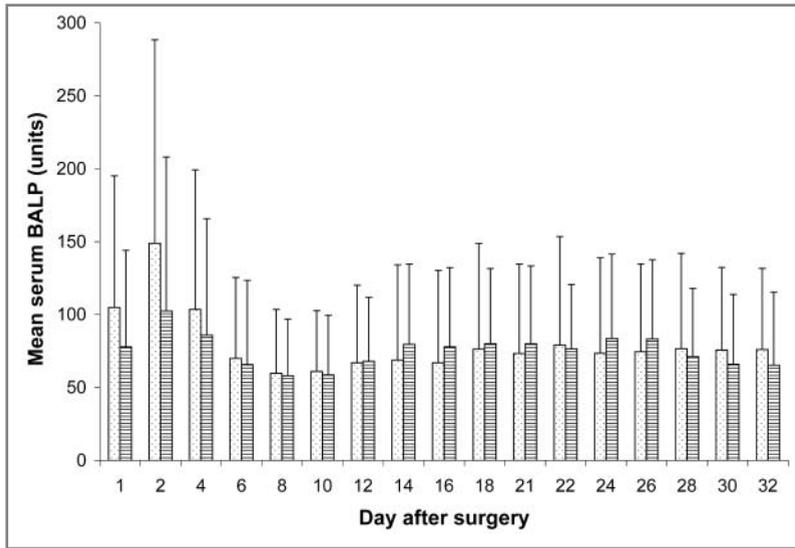
Figure 6—Mean ± SD serum BALP activity for the polycaprolactone-coated (bars with dots) and the polycaprolactone–BMP-2–coated (bars with horizontal lines) groups during the study period. Except for a spike of BALP activity on day 2 for both groups, BALP activity remained relatively constant throughout the study period.

the next 30 days. This slow release would be desirable in many orthopedic environments. The release kinetics were similar to those reported in other studies[23,24] conducted to investigate the elution of BMP-2 from various polymers. It is likely that some of the BMP-2 was on the surface of the coating, and this BMP-2 entered solution almost immediately on immersion in the PBS solution. The BMP-2 that is deeper within the polymer coating was more slowly eluted during the remainder of the experiment. Implant designs or coating methods that increase the surface area-to-volume ratio of the coating would be expected to provide more rapid release of the BMP-2. Coatings that are more textured have higher surface areas and would be expected to release more BMP-2, assuming that the pore size of the textured surface is large enough to allow passage of the BMP-2 molecules. Optimum doses of BMP-2 impregnated in a drug-delivery system in vivo have not been established. Some in vitro experiments have found that higher doses of BMP-2 result in an increased biological effect,[25] whereas others have indicated that BMP-2 enhances cellular function in a non–dose-dependent manner.[26]

The in-vitro study reported here revealed that a polycaprolactone-coated implant may serve as a suitable drug-delivery system for BMP-2. In another study,[8] polycaprolactone was used to create honeycomb-like scaffolds for delivery of BMP-2, and similar results were reported. On the basis of the present study alone, no conclusions can be reached as to how the polycaprolactone–BMP-2 matrix will behave in vivo. However, the subjective histologic analysis suggested that there were problems with polycaprolactone–BMP-2–coated implants as used in this study.

An intracortical gap model was used in this study in an effort to reduce the frictional forces on the polymer coating during screw insertion and to eliminate displacement of the polymer from the implant, which was detected during a preliminary study conducted by members of our laboratory group in which the screws were inserted via standard screw insertion. During that preliminary study, the torque incurred during implantation of the coated implants via standard screw insertion was greater than the bond between the implant and the coating. Thus, the coating was displaced from the surface of the implants. Interestingly, the polycaprolactone–BMP-2 coating separated from the surface of all screws, whereas coatings containing polycaprolactone alone were successfully implanted in some cases without separation of the polymer from the screw. Thus BMP-2 impregnation may weaken polycaprolactone bonding of an implant. The method in the sheep reported here was developed to allow evaluation of intracortical bone-implant interface by use of a bone-implant gap.

Histologically, the amount of new bone formation between the threads of the control screws was greater than that of the polycaprolactone–BMP-2–coated screws. There was little to no new bone formation between screw threads of the polycaprolactone-coated or polycaprolactone–BMP-2–coated implants. These findings suggested that the polycaprolactone coating inhibited growth of bone into the spaces between threads of the implant. We speculate that this was primarily attributable to a barrier effect created by the polymer coating.

Inhibition of bone formation caused by breakdown products of the polycaprolactone is also a possibility. Polycaprolactone is primarily degraded in vivo via hydrolysis to oligomers and monomers, whereas the hydroxyl and carboxyl end groups produce carboxylic acid.[27] In most biological systems, the degradation products remain in low enough concentrations at the local degradation site so that they do not cause biological reactions. However, should the degradation products accumulate locally, they could result in inflammation and adverse reactions.[28]

In the photomicrographs of the bone-screw interface, there was a white area in the peri-implant region around all coated screws (polycaprolactone and polycaprolactone–BMP-2 groups). Although it is possible that the polymer was resorbed over the duration of the experiment, it was likely present at the end of the experiment and was removed during the sectioning process. Because of the sharp margination of the cortical-gap interface, we speculate that there was a barrier effect preventing bone growth onto the screw surface up until the time of screw harvest. We cannot rule out the possibility that biological effects of the coatings inhibited the bony response. However, we know that the coating was intact at the beginning of the experiment because the intact coating was observed as the screws were inserted into the bone.

The implants were not autoclaved because autoclaving temperatures would have melted the polycaprolactone and resulted in its separation from the screws. Sterilization with ethylene oxide also was not used be-

cause adequate degassing times for polycaprolactone have not been established. The surfaces of the implants were disinfected with 95% ethanol and rinsed with sterile saline solution prior to insertion into the bone. To the authors' knowledge, there are no published reports of the effect of ethanol on the activity of BMP-2. It is possible that the ethanol decreased the biological activity of the BMP-2.

A gap between the screw and the leading edge of new bone was detected histologically in all coated screws. Although the physical barrier created between the screw and the bone by the polymer was the likely reason for the lack of new bone adjacent to the implant, biological inhibition by the breakdown products of polycaprolactone cannot be discounted.

Determination of osteonal activity was used to assess whether polycaprolactone and BMP-2 had an affect on osteonal recruitment for this bone trauma technique. Because BMP-2 stimulates new bone formation, we expected that the BMP-2 groups would have an increased number of active osteons. Use of alternating bone markers allowed for temporal assessment to determine the time when the osteons were active. There was an increased number of osteons with uptake of bone marker on day 14 of the study, and this was most pronounced in the BMP-2–coated screws. Increased osteonal activity was expected during the early phase after surgery because osteons are activated following bone trauma.

The overall uptake of bone markers was lower than expected, and several specimens had no evidence of osteonal uptake. The low osteonal uptake is thought to reflect a lower bone metabolism that may have been caused by the age or nutritional status of the sheep.

Dual energy x-ray absorptiometry was performed on the samples to quantitatively measure BMD around the screws. We anticipated that BMP-2 would increase the amount of new bone around the screws and hence increase BMD in the BMP-2 group, as compared with results for the other groups. There was no difference in BMD among groups. These findings are consistent with histologic features of the bone-implant interface. Longer-term study periods may have elucidated differences in changes at the bone-implant interface, but results of other studies[9,10] conducted by use of BMP-2 suggested that a 30-day period was appropriate.

Measurement of peak removal torque is a quantitative assessment of the integrity of the bone-implant interface. The force required to remove the implant is the best overall evaluation of the bone-implant interface. Although not significantly different, the removal torque for the control screws was greater than that for the coated screws (polycaprolactone or polycaprolactone–BMP-2). The coatings (polycaprolactone alone and polycaprolactone–BMP-2) appeared to have a negative effect on the strength of the bone-implant interface during the period of study. The presence of the coating caused a gap between the metal implant and the bone. The polymer coating acted as a weak link at the interface between the coating and the metal screw, which resulted in decreased resistance to torque. Inclusion of BMP-2 within the polymer did not affect peak removal torque.

Significant differences in peak removal torque would likely have been detected had the sample size been larger. A power analysis indicated that a sample size of approximately 20 independent observations/group would have been needed to detect significant differences among the groups. This is considerably more than the 4 samples/treatment group that we used.

The increase in BALP immediately after surgery in response to the bone trauma created was expected. There was no difference detected in the increase of BALP activity, nor the day 0 BALP activity for those sheep that received implants of BMP-coated screws and those that received implants of non–BMP-coated screws.

Other studies have revealed that BMP-2 increases osteoclast[29] and osteoblast[30] activity and increases bone turnover.[29] Furthermore, BALP is used as an indirect biomarker of bone metabolism.[31] On the basis of results of the BMP-2 elution experiment reported here, an extremely small amount of BMP-2 was eluted from the polycaprolactone matrix during the 30-day period. Although optimum dosages for BMP-2 have not been established, it is likely that the BMP-2 concentrations delivered to the bone-implant interface were too minute to achieve a biological effect sufficient to stimulate detectable changes in serum BALP activity. Use of BMP-2 in dogs[15,32,33] and humans[11] can expedite fracture healing; dosages > 1 µg/mL (which was the dosage used in our study) have been used. Our dosage was based on other reports[34–38] in which it was suggested that much lower dosages can be used to achieve clinical effects.

Results of the study reported do not support the use of polycaprolactone–BMP-2 coating of orthopedic implants to increase osseointegration. The polycaprolactone coating may inhibit osseointegration by acting as a physical or chemical barrier to the formation of new bone. Impregnation of polycaprolactone with BMP-2 at the dosage used in this study did not have a beneficial effect on osseointegration.

Variables that had objective, continuous measurements, such as peak removal torque, BMD, and BALP activity, were statistically analyzed. Other variables had subjective, noncontinuous measurements. These included the radiographic analyses, the histomorphometric analysis, and the uptake of bone markers. These data were difficult to analyze without violating many of the rules of the statistical tests, and it was believed that it would be better to merely report the data and provide descriptive interpretations of the results.

Limitations of this study included the low number of sheep in each of the treatment groups and the short interval between implantation and harvesting. It is possible that by increasing the duration of the study and increasing the dose of BMP-2, more bone response may have been detected. To keep the number of experimental subjects to a minimum, multiple screws were inserted in each limb. For this reason, each screw was not truly independent from another because of confounding factors that may have been specific for each sheep. We also placed control screws in another limb of each sheep. This design helped to decrease the number of experimental subjects needed for the experiment and to decrease variation between subjects that had coated screws and control screws. However, we did not account for the possible systemic effects from elution of BMP-2 and therefore did not account for the possibility that

control screws implanted in sheep with BMP-2 screws may have had healing influenced by the growth factor. The finding of inhibited bone function in the presence of polycaprolactone may warrant further research for use of this polymer coating in areas surrounding implants in which bone formation is undesirable, such as situations in which subsequent removal of the implant will be necessary.

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Appendix

Allocation of bone samples for each of 6 sheep to treatment groups and subsequent analyses.

			Sample location		
Sheep No.	Limb region	Treatment	Proximal	Middle	Distal
1	Metacarpus	Control	PRT	PRT	HM-BMD
	Metatarsus	PCL	PRT	PRT	HM-BMD
2	Metacarpus	PCL–BMP-2	HM-BMD	PRT	HM-BMD
	Metatarsus	Control	HM-BMD	PRT	HM-BMD
3	Metacarpus	Control	HM-BMD	HM-BMD	PRT
	Metatarsus	PCL	HM-BMD	HM-BMD	PRT
4	Metacarpus	Control	PRT	HM-BMD	PRT
	Metatarsus	PCL–BMP-2	PRT	HM-BMD	PRT
5	Metacarpus	Control	PRT	HM-BMD	HM-BMD
	Metatarsus	PCL–BMP-2	PRT	HM-BMD	HM-BMD
6	Metacarpus	PCL	HM-BMD	PRT	HM-BMD
	Metatarsus	Control	HM-BMD	PRT	HM-BMD

The right metacarpus and right metatarsus of each sheep were randomly assigned to receive polycaprolactone-coated (PCL), polycaprolactone–BMP-2–coated (PCL–BMP-2), or uncoated (control) screws (3 samples/limb; a proximal, middle, and distal screw). Each sample was analyzed for peak removal torque (PRT) or submitted for histomorphologic analysis and BMD testing (HM-BMD). Identical analyses were performed on corresponding samples from ipsilateral limbs of each sheep so that a control sample was matched with each experimental sample.