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Evaluation of rhBMP-2 With an OPLA Carrier in a Canine Posterolateral (Transverse Process) Spinal Fusion Model

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Study Design. Posterolateral L4–L5 transverse process fusions were done on 14 adult beagles. Six were implanted with recombinant human bone morphogenetic protein-2 carried by open-cell polylactic acid polymer delivery vehicle. Six received autogenous iliac bone graft. Two received carrier alone. Eleven were killed 3 months after implantation. One in each group was maintained for 8 months.

Objectives. To compare recombinant human bone morphogenetic protein-2 and open-cell polylactic acid polymer with autogenous iliac bone for inducing transverse process fusion in the canine by 3 months and to determine whether transverse process decortication and implantation of carrier alone causes spontaneous transverse process fusion in the canine.

Summary of Background Data. Recombinant human bone morphogenetic proteins have healed segmental long bone defects in several models. They have induced interlaminar and facet fusions in canines. Interlaminar and facet fusions have occurred after sham decortications in canines. Recombinant human bone morphogenetic protein-2 has not been evaluated for transverse process fusion in canines. Transverse process fusion is a preferred clinical method for achieving posterior lumbar fusion.

Methods. Fusion sites were evaluated by serial computed tomography scans. After the dogs were killed, explanted spines were subjected to manual testing, mechanical testing, high resolution radiography, and histologic analysis.

Results. One hundred percent of recombinant human bone morphogenetic protein-2-implanted sites had solid transverse process fusion by 3 months according to all measures. No autografted sites were fused at this interval. Osseous bridging of posterolateral gutters occurred in the recombinant human bone morphogenetic protein-2-implanted sites after 2 months, the earliest radiographic measure. None of the carrier-only sites showed bone formation.

Conclusions. Recombinant bone morphogenetic protein-2 carried by open-cell polylactic acid polymer is superior to autogenous iliac bone for producing radiographically and mechanically solid transverse process fusions in canines by 3 months. Spontaneous transverse process fusion does not occur in canines after decortication and open-cell polylactic acid polymer implantation. [Key words: bone induction, bone morphogenetic protein, canine, polylactic acid polymer, posterolateral fusion, recombinant human bone morphogenetic protein-2] **Spine 1995;20:2669–2682**

Posterolateral transverse process fusion (TPF)is a frequently used method for achieving primary lumbar intersegmental arthrodesis.[13] Autogenous corticocancellous bone chips from the iliac crest, combining osteogenic, osteoconductive, and osteoinductive properties, are currently the most successful grafting material.[15,18] The incidence of pseudarthrosis with use of autogenous bone has been reported as high as 40% for multilevel fusions.[8]

Additional disadvantages with iliac crest graft relate to the adjuvant procedure required to procure the graft. Younger and Chapman noted minor complications such as harvest site pain, hypersensitivity, and buttocks anesthesia in 20.6% of patients and major complications such as deep infections and vascular injuries in 8.6% of patients.[7,12,39]

Fresh, frozen, lyophilized, and demineralized bone matrix from allogeneic sources are used as alternatives or supplements to autogenous bone.[21,22,28] These preparations are impaired by lower osteogenic capacity, higher resorption rate, larger host immunologic response, and reduced revascularization.[14] There are con-

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cerns regarding the ability of alloimplants to transmit infectious agents, including the HIV.[3,5]

Considerable attention has been directed toward development of suitable biosynthetic bone graft substitutes. By showing matrix-induced bone formation *de novo* in extraskeletal sites in mammals in 1965, Urist suggested the presence of osteoinductive proteins that effect differentiation of undifferentiated mesenchymal cells to osteoprogenitor cells.[29] Subsequent purification techniques isolated an osteoinductive, low molecular weight bone morphogenetic protein from insoluble bone matrix gelatin.[16,17,24,27,32,33] More recently, human complementary DNA clones corresponding to polypeptides contained in a highly purified bone morphogenetic protein preparation have been isolated and expressed.[26,35–37]

Recombinant human bone morphogenetic proteins (rhBMPs) have regenerated bone successfully across segmental long bone defects in animals.[6,38] Composite implants containing rhBMPs have achieved interlaminar and apophyseal joint spinal fusion in the canine.[7,23] Inductive proteins have not been tested in a TPF application in the canine. The purpose of the present study was to test the hypothesis that fusions produced by recombinant human bone morphogenetic protein-2 (rhBMP-2) with an open-cell polylactic acid polymer (OPLA) delivery vehicle are mechanically and radiographically superior to those produced by iliac crest bone in the canine transverse process fusion model.

Materials and Methods

Design. Fourteen mature female beagles were assigned randomly either the rhBMP-2–OPLA composite implant (n = 6), the autogenous iliac bone implant (n = 6), or the OPLA only implant (n = 2) for a single level L4–L5 posterolateral TPF without instrumentation or immobilization. During the procedure, the surgeon was not aware of the assigned implant until the time of implantation. All procedures were performed by the same surgeon to assure consistency. All animals were killed 3 months after surgery except for one in each group. These three animals were retained for long-term follow-up evaluation (8 months).

Materials. The OPLA delivery vehicles were prepared from D,D-L,L-polylactic acid (OPLA) to form a network of incomplete and interconnected voids (93% of total volume = void space) optimized to duplicate the architecture of cancellous bone. The pure OPLA (2.2 cc, volume) was sterilized via 2.9 Mrad gamma irradiation and provided as 12.0 mm × 6.0 mm × 30.0 mm rectangular strips (supplied by Sofamor Danek, Memphis, TN; developed and manufactured by THM Biomedical, Duluth, MN).

The rhBMP-2 (manufactured and supplied by Genetics Institute, Andover, MA) was provided in freeze-dried form and reconstituted during surgery with sterile water. One cc of solution containing 2.3 mg of rhBMP-2 was combined with each OPLA strip to produce the composite implant. The solution was drip applied to both 360 mm^2 surfaces of each implant and was absorbed completely.

Autogenous bone was harvested from both iliac crests in sufficient volume to implant at least 2.2 cc per side. The graft was morselized into 0.1- to 0.4-cc chips to maximize the surface area of exposed cancellous bone.

Subjects. Mature female beagles, weighing between 7.7 and 13.0 kg (average, 10.98 kg) and with an age range of 1.1–2.6 years (mean age, 1.6 years), were used for evaluation. The animals were screened for systemic disease and conditioned for at least 1 week before inclusion in the study.

Surgical Procedure. Single-level posterolateral TPFs of the L4–L5 segmental level were done. After halothane anesthesia and endotracheal intubation, each canine was placed prone on the operating table, and the posterior lumbar region was shaved, prepared, and draped in sterile fashion. A limited midline incision was made over the L4 and L5 spinous processes and dissection carried to the dorsolumbar fascia. A localization radiograph was taken to ensure exposure of only the appropriate level. The spinous processes, lamina, and transverse processes of the L4 and L5 segments and the intervening apophyseal joints were cleaned completely of soft tissues. Care was taken to avoid inadvertent exposure of adjacent facet joints or transverse processes. The appropriate transverse processes, lamina, and facet joints were decorticated with a power burr (Dremel Moto-Tool, Model 395) until punctate bleeding was observed. The entire wound was irrigated thoroughly to remove any residual osseous debris.

At this time, the surgeon was notified of the randomly assigned treatment. Accordingly, either the rhBMP-2–OPLA composites, the OPLA implants, or the morselized iliac bone were placed into the right and left posterolateral gutters spanning the transverse processes of L4 and L5. For canines assigned to the OPLA only or the rhBMP-2–OPLA treatments, each implant was placed into the host bed such that it had complete contact with decorticated transverse processes. For canines assigned to the autogeneous bone graft treatment, an incision was made over both iliac crests, and the inner and outer tables of the posterior crests were exposed. Tricortical segments of graft were retrieved from each crest and morselized with a rongeur. This graft was placed abutting the decorticated transverse processes. No graft was placed along the facet joints or lamina.

Routine closure of the dorsolumbar fascia, subcutaneous tissue, and skin was performed. Parenteral antibiotics (enrofloxacin, 2.5 mg/kg or cefazolin, 2.0 mg/kg) were given during and after surgery for at least 72 hours. No dressings or braces were applied after surgery.

Computed Tomography Evaluation. Under nembutal anesthesia, computed scans were obtained 2 and 3 months after surgery in all animals and at 8 months in the long-term follow-up canines.

Sections of the fusion site were qualitatively and quantitatively graded by a radiologist blinded to the treatments. The parameters of evaluation included the presence or absence of bridging of the intertransverse process space and characterization of density as either isodense or hypodense to host bone.

Sacrifice. Eleven canines were killed 3 months after surgery, and three canines were killed 8 months after surgery. Eutha-

nasia was achieved by administering 0.22 mL/kg of Euth-6 Veterinary Solution (Western Medical Supply Co., Arcadia, CA). The lumbar spine (L2–L7) was explanted after the dogs were killed.

Manual Testing. After explantation, the L4–L5 motion segment was tested manually in the sagittal and coronal planes. The presence of any motion in either plane was considered a nonfusion.

High Resolution Radiographic Evaluation. Using the Faxitron imaging device (Field Emission Corporation, McMinnville, OR), a high resolution radiograph was taken of the explanted lumbar spine in the coronal plane after cleaning of soft tissues. (Film: Kodak Industrex Film, 50 cm − 13 cm × 18 cm, #507-2392, exposure ~30 MA @ 3 min). A radiologist blinded to the treatment rated the L4–L5 intertransverse process space for the presence of complete bilateral osseous bridging, complete unilateral osseous bridging, new bone formation but incomplete bridging bilaterally, or no new bone formation. A radiographically successful transverse process fusion was defined as complete and uninterrupted osseous bridging of the transverse processes. Facet or interlaminar union was not considered a successful fusion.

Nondestructive Mechanical Testing. The mechanical tests were conducted on autograft and rhBMP-2–implanted spines and consisted of applying manually pure torques and linear loads and measuring the corresponding angular and linear displacements. The measurements were conducted in the following modes: flexion–extension (sagittal plane), lateral bending (coronal plane), and rotation (axial plane). The endplates of each of the functional units were cleaned of any soft tissue. A hole was drilled through each of the vertebral bodies along the anteroposterior axis. Pins were press fit into the holes to ensure rigid fixation to the test fixtures. A "plus sign"-shaped frame was bolted to each of the pins extending through the vertebral body. One of the frames was fixed, whereas the other was left unsupported and free to move. The arms of the frame were aligned with the flexion–extension and lateral bending axes. Pure bending or torsional loads were applied to the unsupported segment of the functional unit through a cable–pulley arrangement. Equal loads were applied to both of the opposing arms using a force handle equipped with strain gauges. A maximum load of 50 N was applied to the system. The maximum load was determined during preliminary tests on nontreated spines to ensure that the levels applied were within the nondestructive range to allow complete elastic recovery of the functional unit. The vertical displacement of the free end of the functional unit was measured using a linear variable differential transformer (LVDT). The angular displacement was measured using a rotational variable differential transformer (RVDT) through a four bar linkage. Orientation of the functional unit defined the flexion–extension, lateral bending, and the rotation axes of the applied loads. Three consecutive trials were performed for each mode of testing. The slope of the force–displacement curve at 45 N of applied force was used to calculate the stiffness in each mode.

Histology. Spinal segments from dogs killed 8 months after surgery were placed in 10% formalin immediately after explantation and manual testing. The other spinal motion segments were placed in 10% formalin after manual and mechanical testing were performed. The 8-month rhBMP-2–OPLA-implanted sites underwent decalcified sectioning, and remaining sites underwent undecalcified analyses including microradiography and toluidine blue-O and basic fuchsin staining. The implants and tissues were dehydrated sequentially in 70% alcohol for 2–3 days, 95% alcohol for 2–3 days, two changes of 100% alcohol for 2–3 days each, and xylene for 1 day. S/P Decalcifying Solution (E.D.T.A., Baxter, Riverdale, NJ) diluted in hydrochloric acid was used to demineralize the decalcified specimen until transparent, and it was embedded in Paraplast Plus (Fisher, Pittsburgh, PA). The undecalcified implants were embedded in methylmethacrylate. After polymerization was complete, the blocks were sectioned on a diamond saw (Buehler Isomet, Lake Bluff, Il) to produce at least 10 sections of an approximate thickness of 100–200 μm. One side of the bisected level was sectioned in the sagittal plane, whereas the other side was sectioned in the transverse plane. This orientation was randomized for all spinal levels.

Undecalcified sections of the explants from the present study were radiographed using Copper k-alpha radiation at 20 kV and 30 mA using a microradiography unit (Kristalloflex-2, Siemens, New York, NY) and spectroscopic film (Kodak, Rochester, NY). A custom-made camera with an extension tube measuring 22.9 cm in length was used to obtain high resolution microradiographs. The thickness of the sections was measured with a metric micrometer (Fowler, Newton, MA) to determine the exposure time. Sections were exposed for 12.5 minutes for each 100 μm of thickness. The samples were placed on the spectroscopic plates, and the plates were placed on a rectangular holder. A piece of latex was placed over the plate and holder, and a vacuum applied to the holder, holding the sections in place and preventing the formation of shadows on the plates. The cassette assembly was inserted into the camera mounted on the x-ray unit and exposed to the x-radiation as described. These plates were developed, fixed, and analyzed for ossification using standard optical microscopy. Staining was performed on the undecalcified sections after microradiographs were produced to determine the histologic and cytologic response to the OPLA carrier and to OPLA–rhBMP-2 and to autograft. Differential staining with basic fuchsin and toluidine blue-O was used to enable histologic differentiation. After staining, all sections were read by an orthopedic histopathologist in a blinded fashion. Undecalcified stained sections and microradiographs were evaluated for bone graft resorption and replacement by creeping substitution, the host response to the implant materials including the presence or absence of an inflammatory response to the carrier, and the amount and quality of bone induced by rhBMP-2.

Statistical Analysis. Qualitative manual testing, radiographic, and computed tomography data were analyzed using Fisher's exact test. For the nondestructive mechanical testing data, analysis of variance (BMDP Statistical Software 2V.10, Cary, NC) was applied to the three consecutive measures of motion (within factor of three levels) separately for each mode of testing: sagittal bending, coronal bending, and axial rotation (within factor of two levels). There was one grouping factor (rhBMP-2–OPLA *vs.* autogenous iliac bone).

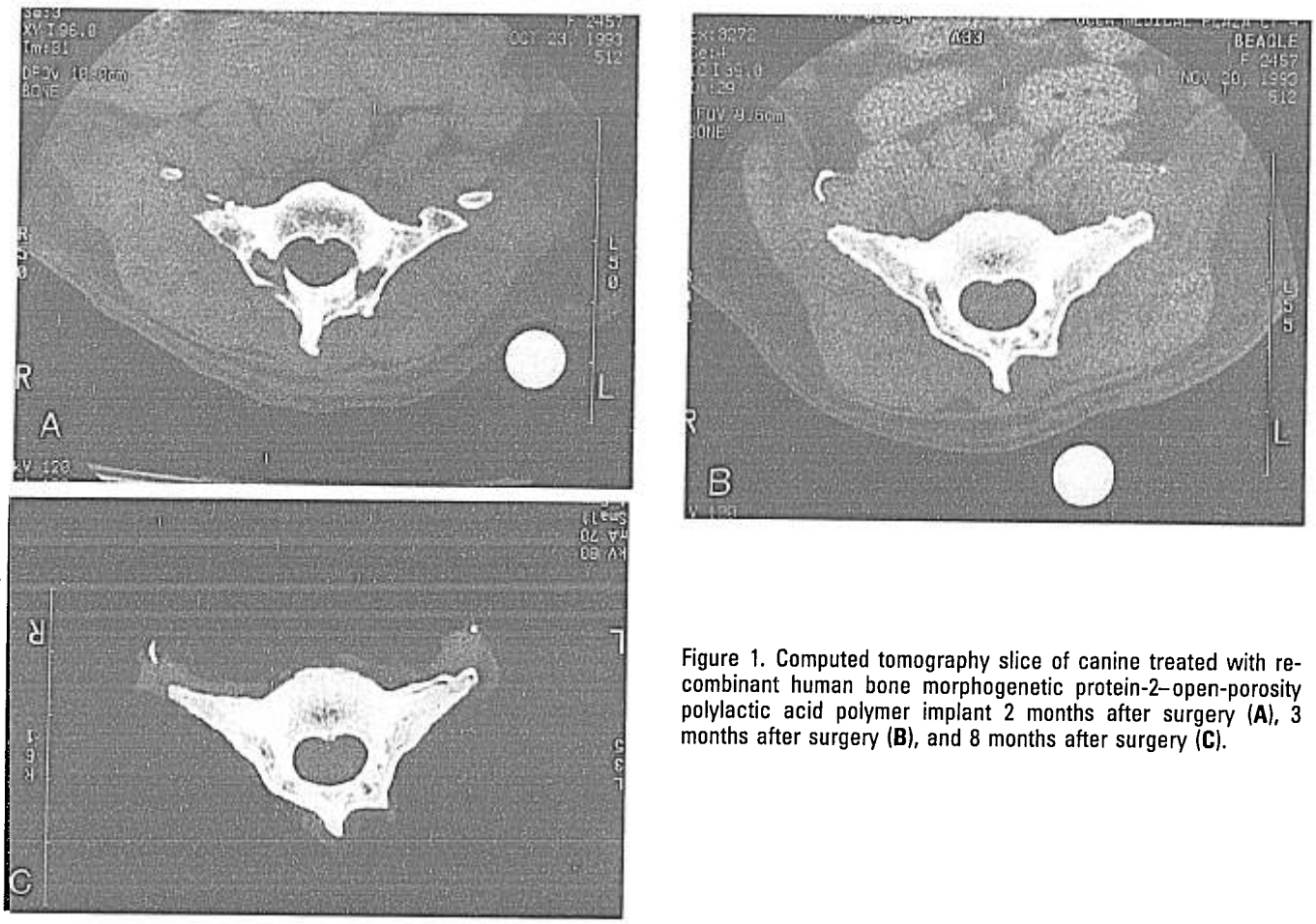

Figure 1. Computed tomography slice of canine treated with recombinant human bone morphogenetic protein-2–open-porosity polylactic acid polymer implant 2 months after surgery (**A**), 3 months after surgery (**B**), and 8 months after surgery (**C**).

Results

Surgery

All animals tolerated the procedures well, and no perioperative deaths occurred. One beagle, implanted with rhBMP-2–OPLA, exhibited immediate bilateral quadriceps weakness and 8 days after surgery was killed and excluded from the study. The neurologic deficit was attributed to injury to the extraspinal lumbar roots during surgical dissection. This canine was replaced to maintain study design.

The mean age and weight at the time of surgery were similar between canines implanted with rhBMP-2–OPLA and those implanted with autograft (age, 1.56 yr *vs.* 2.00 yr; T = 1.77; $P > 0.05$; and weight, 10.78 kg *vs.* 11.49 kg, T = 0.66, $P > 0.05$; canines implanted with OPLA only had a mean age of 1.1 yr and weight of 10 kg).

Radiographic Evaluation

Computed tomography images obtained 2 months, 3 months, and 8 months after surgery for rhBMP-2–OPLA-treated spines are depicted in Figure 1 and for autograft treated spines in Figure 2. Computed tomography scans obtained 2 months after surgery revealed abundant bone formation with bilateral osseous bridging of the intertransverse process space in all six animals containing the rhBMP-2–OPLA composite implant (Figure 1A). There was no evidence of recorticalization of the fusion mass by this time. In contrast, computed tomography scans obtained in canines implanted with autogenous iliac crest bone graft showed minimal new bone formation and some evidence of graft resorption (Figure 2A). The canines implanted with OPLA only had no new bone formation. Repeat computed tomography scans obtained 3 months after surgery revealed in the rhBMP-2-implanted group the formation of new cortices and increased radiodensity of the fusion mass (Figure 1B). Eighty-three percent (five of six) of the experimental (rhBMP-2–OPLA) specimens were graded as complete osseous union with new bone qualitatively isodense to host bone (Table 1). Seventeen percent (one of six) were graded as complete osseous union with new bone hypodense to host bone. None (zero of six) of the autogenous bone-implanted sites exhibited complete intertransverse process osseous bridging (Figure 2B). These differences were significant (Fisher's exact test, $P < 0.003$). The canines implanted with OPLA only

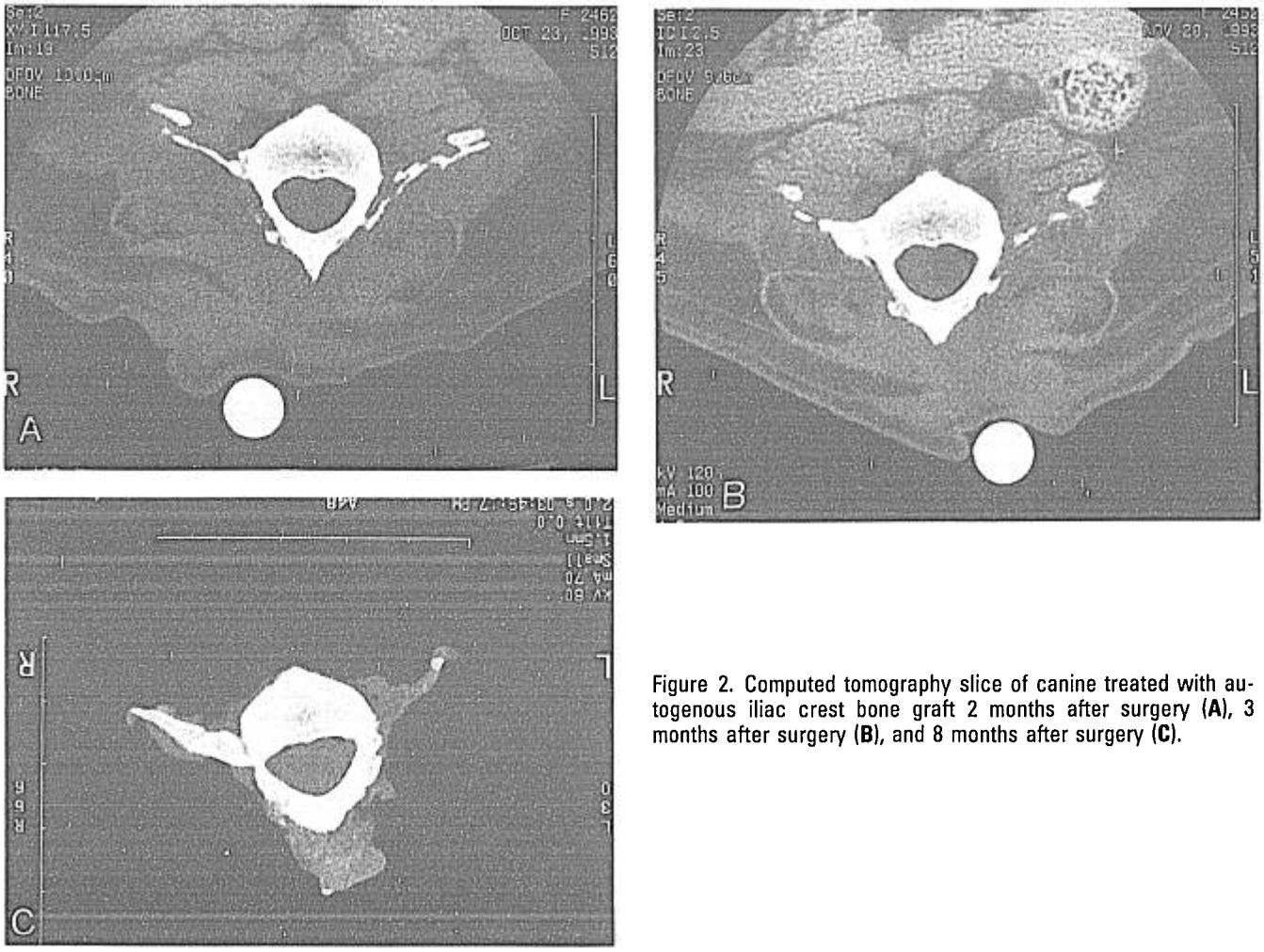

Figure 2. Computed tomography slice of canine treated with autogenous iliac crest bone graft 2 months after surgery (**A**), 3 months after surgery (**B**), and 8 months after surgery (**C**).

showed minimal bone at decorticated surfaces but no evidence of bone formation in the posterolateral gutters.

High resolution radiographs for rhBMP-2–OPLA-implanted spines are presented in Figure 3 and for autograft implanted spines in Figure 4. By 3 months, high resolution radiographs (Table 2) of each of the rhBMP-2 fusion sites (five of five) showed complete osseous union of the L4–L5 intertransverse process space (Figure 3A–E). In the autograft group, none of the implant sites (zero of five) exhibited complete bilateral osseous union (Figure 4 A–E; Fisher's exact test, $P < 0.003$). A unilateral pseudarthrosis was noted in one specimen. There was no new bone along the intertransverse process space in the canine implanted with OPLA only.

High resolution radiographs (Table 2) of the 8-month follow-up explanted spines revealed abundant bone formation in the rhBMP-2-implanted sites (Figure 3F) compared with a unilateral fusion in the autograft-implanted specimen (Figure 4F). There was no new posterolateral bone in the canine implanted with OPLA only at this time.

Computed tomography imaging of the rhBMP-2-implanted site at 8 months revealed abundant bone formation in the axial plane and establishment of a dense peripheral cortex contiguous with native cortical bone (Figure 1C). Computed tomography imaging of the autograft-implanted site at this time point confirmed a unilateral intertransverse process union but showed

Table 1. Computed Tomography

3-Month Cohort (n = 14)	rhBMP-2/OPLA n = 6	Autograft n = 6	OPLA n = 2
Bilateral bridging with isodense bone	5	—	
Bilateral bridging with hypodense bone	1	—	—
Unilateral bridging	—	—	—
Incomplete bridging	—	2	—
No new bone	—	4	2
8-Month Cohort (n = 3)	**rhBMP-2/OPLA n = 1**	**Autograft n = 1**	**OPLA n = 1**
Bilateral bridging with isodense bone	1	—	—
Bilateral bridging with hypodense bone	—	—	—
Unilateral bridging	—	1	—
Incomplete bridging	—	—	—
No new bone	—	—	1

rhBMP-2 = recombinant human bone morphogenetic protein-2. OPLA = open-cell polylactic acid polymer.

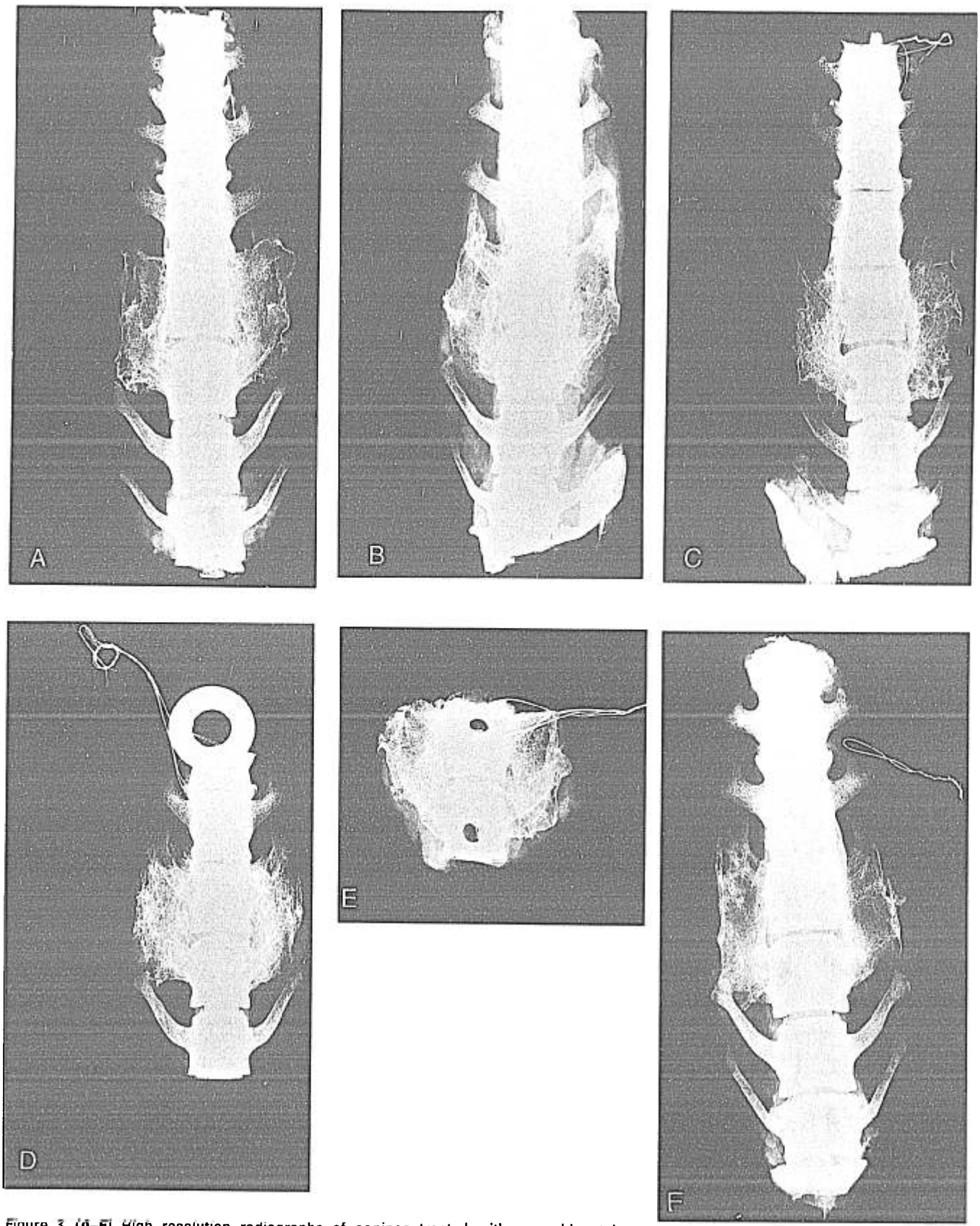

Figure 3. (A–E) High resolution radiographs of canines treated with recombinant human bone morphogenetic protein-2–open-cell polylactic acid polymer (rhBMP-2–OPLA) implants killed 3 months after surgery (n = 5). (F) High resolution radiographs of canine treated with rhBMP-2–OPLA implants killed 8 months after surgery (n = 1).

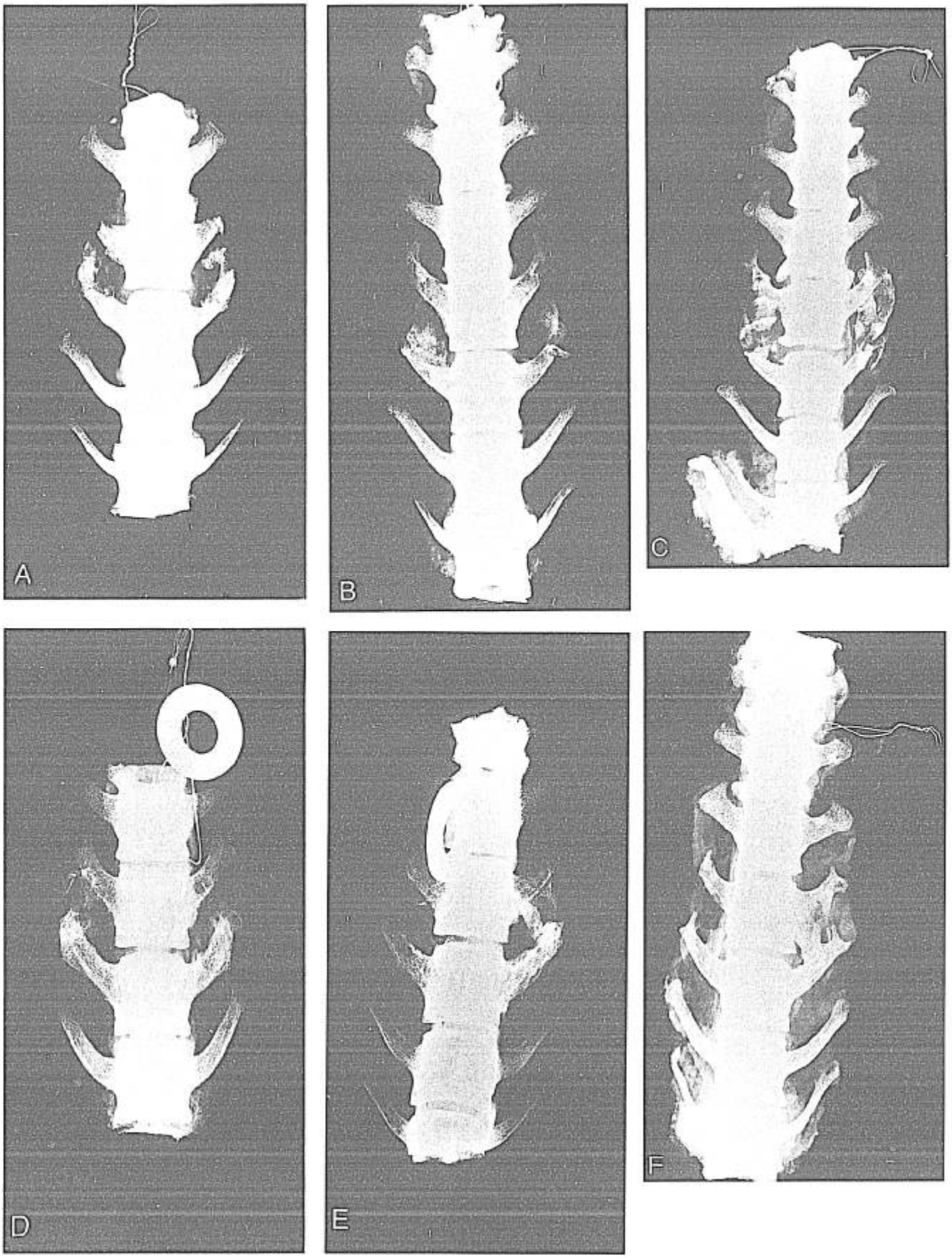

Figure 4. **(A–E)** High resolution radiographs of canines treated with autogenous iliac crest bone graft killed 3 months after surgery (n = 5). **(F)** High resolution radiographs of canine treated with autogenous iliac crest bone graft killed 8 months after surgery (n = 1).

Table 2. High Resolution Radiographs

3-Month Cohort (n = 11)	rhBMP-2/OPLA n = 5	Autograft n = 5	OPLA n = 1
Complete bilateral osseous bridging	5	—	—
Complete unilateral osseous bridging	—	—	—
Incomplete bridging	—	5	—
No new bone formation	—	—	1
8-Month Cohort (n = 3)	**rhBMP-2/OPLA n = 1**	**Autograft n = 1**	**OPLA n = 1**
Complete bilateral osseous bridging	1	—	—
Complete unilateral osseous bridging	—	1	—
Incomplete bridging	—	—	—
No new bone formation	—	—	1

rhBMP-2 = recombinant human bone morphogenetic protein-2. OPLA = open-cell polylactic acid polymer.

interrupted bone formation on the opposite side (Figure 2C).

Manual Testing and Gross Examination

In the specimens killed after 3 months, all of rhBMP-2–OPLA composite implant sites exhibited gross absence of motion to manual testing (five of five rated fused). Conversely, all autogenous graft implant sites exhibited motion to manual testing (zero of five rated fused; Fisher's exact test, $P < 0.003$). In the 8-month explanted specimens, the rhBMP-2 and the autograft sites were rated fused. Both spines implanted with OPLA only exhibited intersegmental motion and were rated not fused.

Gross inspection of rhBMP-2-implanted spines revealed exuberant bone formation along the posterolateral gutters. The thickness of the osseous mass was several magnitudes greater than the thickness of individual transverse processes or lamina. The coronal width of the fusion mass typically spanned the entire extent of the transverse process to the base of the spinous process. Occasional osseous extensions several millimeters in length were noted cephalad to the L4 transverse processes but did not extend to adjacent levels. There were no macroscopic differences within the rhBMP-2 group between 3- and 8-month follow-up evaluations.

Fusion sites containing autograft showed residual fragments of cortical graft with callus along the fragments and decorticated surfaces. The volume of new bone within the fusion bed was considerably less.

Sites containing OPLA only showed minimal bone on decorticated surfaces and along the facet joints but no new bone in the posterolateral gutters.

Mechanical Testing

Mechanical testing was performed on specimens, regardless of the presence or absence of intertransverse bridging, to incorporate contributions of potential facet joint arthrodesis. The three sequential measures were averaged within each mode for each specimen. Specimens treated with rhBMP-2 (n = 5) showed more stiffness ($P < 0.004$) and less displacement ($P < 0.0003$) than the autograft treated specimens (n = 5) to sagittal bending. Axial torque testing exhibited similar results for stiffness ($P < 0.001$) and displacement ($P < 0.0006$). Coronal bending showed similar results for displacement ($P < 0.02$). The results of mechanical testing are summarized in Tables 3 and 4 and in Figure 5.

Table 3. Mean Stiffnesses

	BMP	Autograft	Difference (%)
Flexion (N/cm)	208.2	63.2	229.4*
Extension (N/cm)	150.6	91.0	65.5*
Right bending (N/cm)	129.8	76.5	69.7
Left bending (N/cm)	114.6	95.6	19.9
Right torque (nm/deg)	2.92	0.82	256.1*
Left torque (Nm/deg)	4.15	1.05	295.2*

* Significant.
BMP = recombinant human bone morphogenetic protein-2.

Histologic Examination

Recombinant Human Bone Morphogenetic Protein-2–Open-Cell Polylactic Acid Polymer. Histologic analysis of the stained undecalcified sections from canines implanted with rhBMP-2–OPLA and killed 3 months after surgery revealed a graft site abundantly populated with woven and lamellar bone trabeculae in a cancellous pattern (Figure 6A–C). Some sections revealed a remodeled cortex consisting of dense coarse compact bone, whereas sections from other canines revealed little-to-no cortical remodeling. Transverse processes were bridged with osteopenic trabeculae of woven and lamellar bone. Few secondary osteons were observed. Islands of osteopenic cancellous bone interspersed with fibro-fatty connective tissue surrounded remnants of the OPLA polymer. Fatty marrow was observed in the intertrabecular spaces. Remnants of the OPLA polymer were surrounded by woven and lamellar bone trabeculae (Figure 6D). A low grade chronic inflammatory response was observed.

Table 4. Mean Displacements

	BMP	Autograft	Difference (%)*
Flexion (cm)	0.20	1.00	−80.0
Extension (cm)	0.28	1.04	−73.1
Right bending (cm)	0.34	1.01	−66.3
Left bending (cm)	0.38	0.97	−60.8
Right torque (deg)	0.29	1.72	−83.1
Left torque (deg)	0.29	1.39	−79.1

* All significant.
BMP = recombinant human bone morphogenetic protein-2.

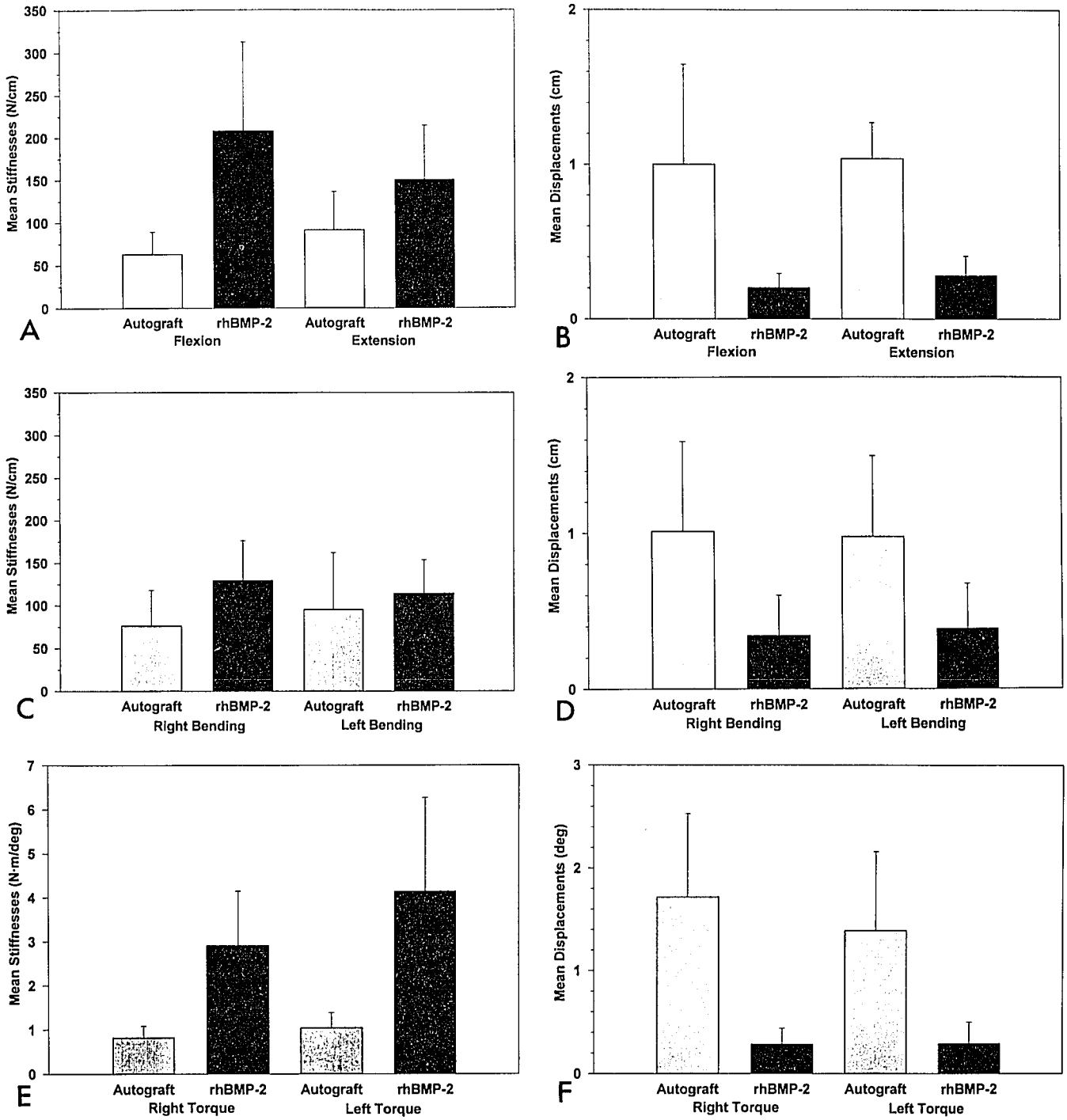

Figure 5. **(A)** Comparison of mean stiffness and standard deviation between canines treated with recombinant human bone morphogenetic protein-2–open-porosity polylactic acid polymer (rhBMP-2–OPLA) and autogenous iliac crest bone graft in flexion and extension. **(B)** Comparison of mean displacement and standard deviation between canines treated with rhBMP-2–OPLA and autogenous iliac crest bone graft in flexion and extension. **(C)** Comparison of mean stiffness and standard deviation between canines treated with rhBMP-2–OPLA and autogenous iliac crest bone graft in right bending and left bending. **(D)** Comparison of mean displacement and standard deviation between canines treated with rhBMP-2–OPLA and autogenous iliac crest bone graft in right bending and left bending. **(E)** Comparison of mean stiffness and standard deviation between canines treated with rhBMP-2–OPLA and autogenous iliac crest bone graft in right torque and left torque. **(F)** Comparison of mean displacement and standard deviation between canines treated with rhBMP-2–OPLA and autogenous iliac crest bone graft in right torque and left torque.

This hypocellular response consisted of round cells and multinucleated giant cells accompanied by fibrous connective tissue. This reaction was far less than the reaction observed when OPLA was used alone. There was an uninterrupted bridge of *de novo* bone in the space between the transverse processes on sagittal sections of all five canines implanted with rhBMP-2–OPLA and killed 3 months after surgery.

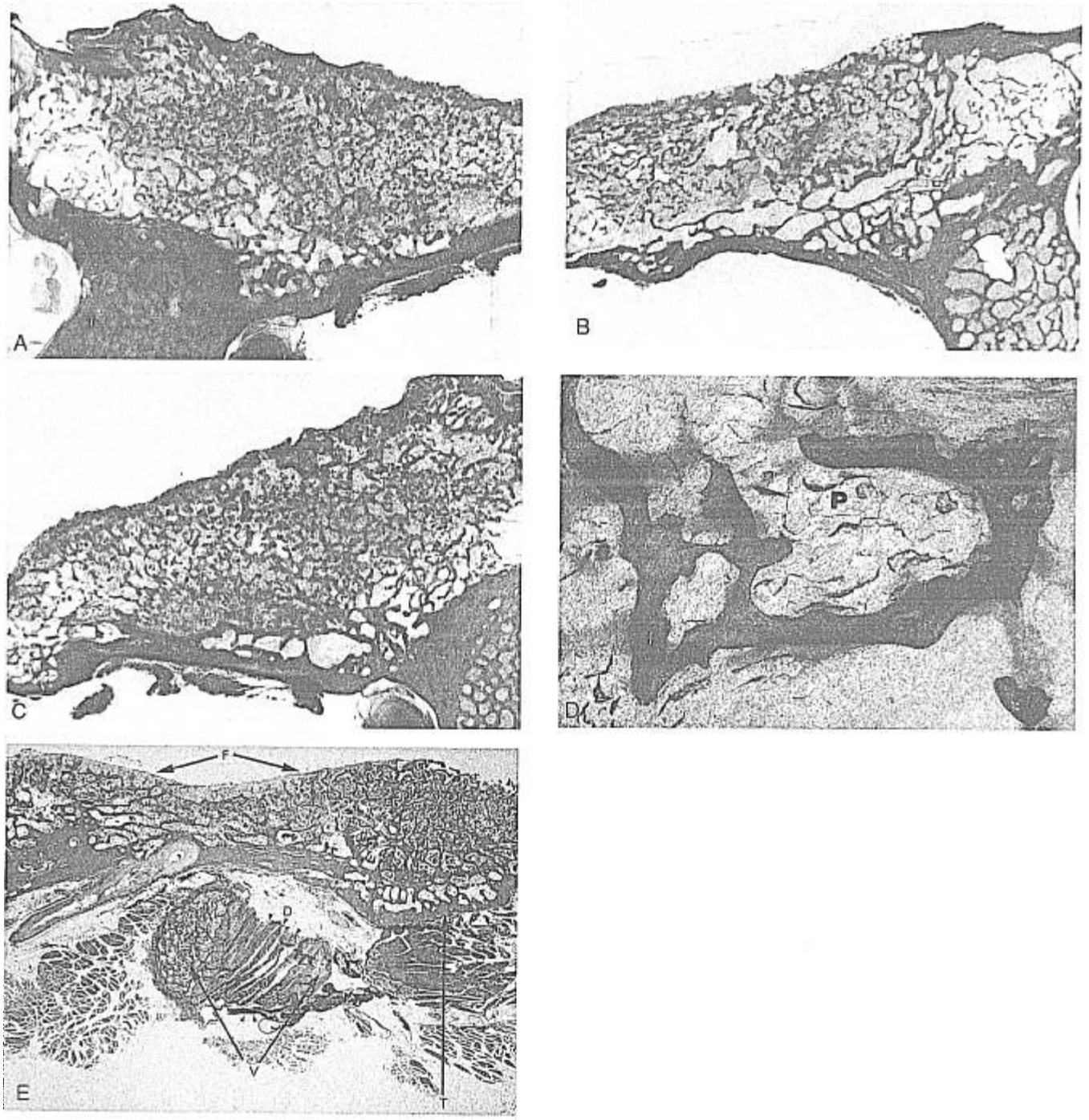

Figure 6. **(A)** Macroscopic view of a stained undecalcified transverse section from a canine implanted with recombinant human bone morphogenetic protein-2–open-porosity polylactic acid polymer (rhBMP-2–OPLA) and killed 3 months after surgery. Abundant *de novo* woven and lamellar bone as small trabeculae are arranged into a cancellous pattern in the graft area (grayish appearance). The cortex has been remodeled with dense coarse compact bone. **(B)** Macroscopic view of a stained undecalcified transverse section from a canine implanted with rhBMP-2–OPLA and killed 3 months after surgery; the graft onlay site shows loosely but uniformly distributed woven bone trabeculae of osteopenic character in a sparse cancellous structure. Little-to-no cortical remodeling can be seen. **(C)** Macroscopic view of a stained undecalcified transverse section from a canine implanted with rhBMP-2–OPLA and killed 3 months after surgery. Abundant *de novo* woven and lamellar bone as small trabeculae are arranged into a cancellous pattern in the graft area (grayish appearance). Some cortical remodeling can be seen. **(D)** Micrograph of a stained undecalcified section from a canine implanted with rhBMP-2–OPLA and killed 3 months after surgery showing woven and lamellar bone trabeculae surrounding a remnant of the OPLA polymer (P). Original magnification = 200 ×). **(E)** Macroscopic view of a stained decalcified sagittal section from a canine implanted with rhBMP-2–OPLA and killed 8 months after surgery. A fusion mass (F) consisting of lamellar coarse and fine trabeculae extends from the inferior to superior vertebral body in the medial part of the intertransverse space. Disk D = disk. V = vertebral bodies. T = transverse processes.

In the canine killed 8 months after surgery (Figure 6E), cancellous lamellar bone in a contiguous pattern spanned the intervertebral space in the sagittal sections. Abundant marrow was found in the intertrabecular spaces. No evidence of the polymeric carrier or chronic inflammatory response was observed at 8 months.

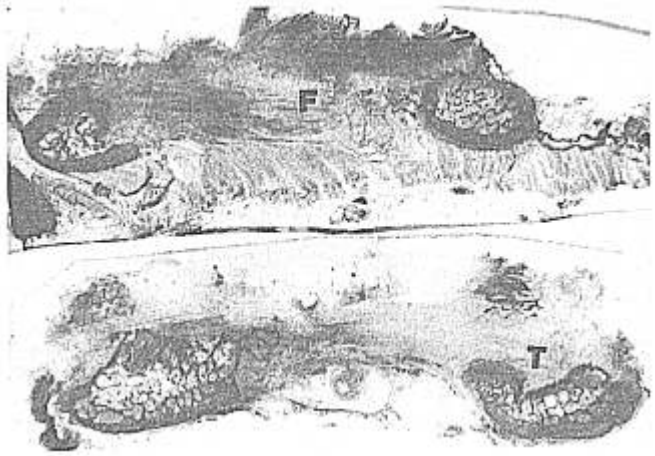

Figure 7. Macroscopic view of two stained undecalcified sagittal sections from a canine implanted with autograft and killed 3 months after surgery. Some bone remodeling is observed on the dorsal surfaces of the decorticated lamina of the transverse processes (T). Only fibrous tissue (F) is observed in the intertransverse space.

Autograft. Histologic analysis of the stained undecalcified sections from the canines treated with autograft revealed a mosaic of fibrovascular tissue that was devoid of bone (Figure 7). In the intertransverse process space, most or all of the autograft was resorbed without concomitant deposition of new bone. Isolated fragments of autograft showed Howship's lacunae with osteoclastic resorption. Bone remodeling was present but was limited to the dorsal surfaces of the decorticated lamina. None of the canines implanted with autograft and killed 3 months after surgery had bridging with *de novo* bone in the intertransverse process space.

Open-Cell Polylactic Acid Polymer. Histologic analysis of stained decalcified sections from the canine implanted with OPLA only and killed 3 months after surgery revealed multiple lobulated vacant spaces (presumably remnants of the polymeric carrier) with fibrous septate walls in the implant site. The fibrous septations were populated with a plethora of multinucleated giant cells, histiocytes, and pyknotic mononuclear round cells characteristic of a foreign body reaction. There was no evidence of bridging in the two canines implanted with OPLA only (3 or 8 months after surgery).

Discussion

Urist showed matrix-induced bone formation in 1965 and reported that perivascular mesenchymal cells, when in contact with lyophilized bone treated with 0.6 N hydrochloric acid, consistently differentiated into cartilage and bone.[29–31] He observed that newly formed woven bone subsequently was resorbed, remodeled, and replaced by a spherical shell of lamellar bone encasing functional bone marrow. Cunningham and Reddi[10] have characterized this phenomenon as a sequential cascade of interrelated events parallel to those occurring during endochondral ossification. The components of matrix-induced bone development include chemotaxis and binding of mesenchymal cells to matrix, proliferation of cells, and differentiation of cartilage, bone, and marrow.

Wang et al[34] in 1988, using SDS-polyacrylamide gel electrophoresis as a final step, retrieved a protein preparation representing a 300,000-fold purification from the initial bone extract. Roughly 20 mg of protein with inductive activity were obtained from 10 kg of intact bone. Subsequently, Wozney et al[37] derived the amino acid sequence of proteins contained in this highly purified preparation and isolated human complementary DNA clones. Many of the recombinantly expressed bone morphogenetic proteins were capable independently of inducing bone formation *in vivo*.[9,11,35]

At least 11 rhBMPs have been identified, and all are believed to have been conserved for roughly 600 million years.[1,36]

Applied Preclinical and Clinical Studies

Nilsson et al[25] in 1986 evaluated the ability of a partially purified bovine BMP to heal segmental ulnar diaphyseal defects in the canine. Callus, consisting of fibrocartilage, cartilage, and woven bone, was noted within 4 weeks, and by 12 weeks, remodeling to lamellar bone had occurred.

In 1988, Johnson et al reported that 12 patients with established nonunions of the femoral diaphyseal or metaphyseal–diaphyseal shaft had an average time to union of 4.7 months after implantation of an aggregate of hBMP and bone matrix noncollagenous proteins (50–100 mg of hBMP–iNCP per implant).[20] An average of 4.3 grafting or fixation procedures were performed before BMP implantation. In all cases, the partially purified, guanidine-extracted protein aggregate was carried in ultrathin gelatin capsules or incorporated in a strip of polylactic/polyglycolic acid (PLA/PGA) copolymer. The same authors evaluated six patients with traumatic segmental defects of the tibia, ranging 3–17 cm. They observed solid union within the defects after implantation of hBMP/iNCP (50–100 mg per implant) carried on a PLA/PGA copolymer with autogenous cancellous grafts and stabilization.[19] Weight bearing was permitted at an average of 5.7 months after surgery. Both studies showed that when used as an adjunct to autograft, partially purified hBMP produced no adverse effects.

Bone morphogenetic protein augmentation of autogenous bone for spinal fusion was reported first by Lovell and Dawson.[23] Partially purified BMP (50 mg per implant) carried on a polylactic acid polymer, when implanted in a lower thoracic intervertebral segment in canines, manifested more rapid fusion rates and produced larger fusion masses than that of spinous process graft alone. Six months after surgery, the polymer was not resorbed fully.

Therapeutic applications of recombinant osteoinductive proteins have been evaluated. Yasko and Lane[38] reported the first orthotopic application of a recombinant BMP using an internally stabilized rat femoral segmental defect model. Two doses of rhBMP-2 (1.4, 11.0 mg) combined with demineralized, inactive, rat-bone matrix were implanted in 5 mm femoral defects and compared with matrix-only implants. Bone formation in the high-dose group was noted as early as 7 days after surgery, and evidence of radiographic union was noted as early as the third week. Eight of 10 femora in the high dose group achieved union by 6 weeks, whereas no instances of union were noted in the low dose group, suggesting a dose–response or dose threshold–response relationship of rhBMP-2 to orthotopic formation of bone.

The use of recombinant osteoinductive proteins in the spine was reported recently by Cook et al.[7] Human osteogenic protein-1 (rhBMP-7) delivered by a collagen carrier was used to induce interlaminar and facet fusion in six mongrel canines. Extensive new bone formation with osteogenic protein-1 was noted as early as 6 weeks, and complete fusion was observed 12 weeks after surgery. Although limited data points were available, they observed mechanically superior stiffness at all time points in osteogenic protein-1-induced interlaminar fusions.

The present study has explored the efficacy of rBMP with an open cell polylactic acid polymer in a posterolateral TPF model in the canine. Previous studies had shown that the canine has a propensity to achieve interlaminar and facet fusion in 6 months by decortication alone.[4] Consequently, the transverse process fusion model was selected. It was hypothesized that decortication and implantation of carrier alone would not sufficiently induce osseous bridging across the intertransverse process space by either the 3-month or 8-month time point. This condition would serve consequently as our negative control. There is no available data on the use of autogenous iliac bone graft in a transverse process fusion model in the beagle.

The decision to use a OPLA polymer as the delivery vehicle was, in part, based on its biocompatibility.[2] The porous macrostructure was designed to enhance exposure of the cytokines to mesenchymal cells and to facilitate degradation.

Abundant bone formation and bridging of the intertransverse process space was noted within 2 months of implantation of the rhBMP-2–OPLA composite. By 3 months, the transverse process fusions were radiographically and clinically solid in all rhBMP-2-implanted specimens, whereas none of the autograft-implanted specimens had achieved either radiographic or clinical intertransverse process union.

Computed tomography and high resolution radiography confirmed that by 3 months, the rhBMP-2-induced fusion masses were isodense with host bone and had reestablished a peripheral cortex. These findings were chronologically similar to those seen in previous canine long bone and spinal fusion models.[7,23,25]

By 8 months, the rhBMP-2–OPLA and the autograft-implanted sites had developed clinical TPFs, although, radiographically, the autograft fusion was unilateral. This showed that autogenous iliac bone has the capacity to induce TPF in canines, albeit at a rate inferior to rhBMP-2–OPLA.

Mechanically, more stiffness and less displacement were noted in the rhBMP-2–OPLA-implanted sites. Fewer differences between the rhBMP-2 and autograft groups were noted on lateral bending, and this may reflect the contribution of partial facet arthrodesis. In general, the mechanical stiffness measures of the rhBMP-2-implanted sites were consistent with intertransverse process osseous union.

Canines implanted with OPLA only produced no new bone formation in the intertransverse process space and failed to achieve TPF. This supported the hypothesis that decortication and implantation of noninductive materials would not yield a positive outcome with this model.

Histologic analysis revealed the rhBMP-2–OPLA composite induced mature bone with normal architectural characteristics. Elements of fatty marrow and isolated bone spicules suggested the presence of remodeling. The chronic foreign body response to the rhBMP-2–OPLA composite was far less than the reaction observed when OPLA carrier was used alone. The reason for this is not clear. It may be that the BMPs may cause cells to release cytokines associated with the observed healing instead of the foreign body response associated with the OPLA alone. There is evidence that the OPLA polymer carrier when combined with rhBMP-2 underwent complete degradation somewhere between 3 and 8 months after implantation.

■ Conclusion

Recombinant bone morphogenetic protein-2 of sufficient dose is highly efficacious when combined with OPLA for producing *transverse process* arthrodesis in the canine. The results with this composite implant in this model were superior to those of autogenous iliac crest graft radiographically and mechanically. The TPF model suitably failed to produce positive outcome under sham conditions of decortication and implantation of carrier only.

Potent osteogenic inductive factors may expand greatly the armamentarium of surgeons treating complex spinal disorders. At a minimum, our findings suggest that they may serve as suitable alternatives for autogenous iliac crest bone graft. Current studies are directed toward establishing efficacy of this composite with lower doses of recombinant BMP.

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References

1. Aldinger G, Herr G, Kusswetter W, Reis HJ, Thielemann FW, Holz U. Bone morphogenetic protein: A review. Int Orthop 1991;15:169–77.
2. Alexander H, Parsons JR, Weiss AB, Bajpai PK. Absorbable composites as orthopaedic implants. Transactions of the Society of Biomaterials, San Diego, CA 1985;8:215. Abstract.
3. Buck BE, Malinin TI, Brown MD. Bone transplantation and human immunodeficiency virus: An estimate of risk of acquired immunodeficiency syndrome (AIDS). Clin Orthop 1989;240:129–36.
4. Callewart CC, Kanim LEA, Seeger LL, Dawson EG. Variable fusion rates in the canine model. Presented at 29 Annual Meeting of the Scoliosis Research Society, Portland, Oregon, September 21–24, 1994.
5. Centers for Disease Control. Leads from the MMWR: Transmission of HIV through bone transplantation. Case report and public health recommendations. JAMA 1988;260: 2487–8.
6. Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC, Whitecloud TS III. The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. J Bone Joint Surg [Am] 1994;76:827–38.
7. Cook SD, Dalton JE, Tan EH, Whitecloud TS III, Rueger DC. *In vivo* evaluation of recombinant human osteogenic protein (rhOP-1) implants as a bone graft substitute for spinal fusions. Spine 1994;19:1655–63.
8. Cotler JM, Star AM. Complications of spinal fusions. In: Cotler JM, Cotler HM, eds. Spinal Fusion: Science and Technique. New York: Springer-Verlag, 1990:361–87.
9. Cox KA, Holtrop M, D'Alessandro JS, Wang EA, Wozney JM, Rosen V. Histological and ultrastructural comparison of the in vivo activities of rhBMP-2 and rhBMP-5. J Bone Miner Res 1991;6(Suppl):155.
10. Cunningham N, Reddi AH. Biologic principles of bone induction: Application to bone grafts. In: Habal MB, Reddi AH, eds. Bone Grafts and Bone Substitutes. Philadelphia: WB Saunders, 1992:93–8.
11. D'Alessandro JS, Cox KA, Israel DI, et al. Purification, characterization and activities of recombinant bone morphogenetic protein 5. J Bone Miner Res 1991;6(Suppl):153.
12. Damien CJ, Parsons JR. Bone graft and bone graft substitutes. A review of current technology and applications. Journal of Applied Biomaterials 1991;2:187–208.
13. Frymoyer JM. The Adult Spine. New York: Raven Press, 1991:29.
14. Goldberg VM, Stevenson S, Shaffer JW. Biology of autografts and allografts. In: Friedlander GE, Goldberg, VM, eds. Bone and Cartilage Allografts. Chicago, IL: American Academy of Orthopaedic Surgeons, 1991:3–12.
15. Habal MB, Reddi AH. Bone Grafts and Bone Substitutes. Philadelphia: WB Saunders, 1992:3–8, 376.
16. Hanamura H, Higuchi Y, Nakagawa M, Iwata H, Nogami H, Urist MR. Solubilized bone morphogenetic protein (BMP) from mouse osteosarcoma and rat demineralized bone matrix. Clin Orthop 1980;148:281–90.
17. Heckman JD, Boyan BD, Aufdemorte TB, Abbott JT. The use of bone morphogenetic protein in the treatment of nonunion in a canine model. J Bone Joint Surg [Am] 1991;73: 750–64.
18. Heiple KG, Goldberg VM, Powell AE, et al. Biology of cancellous bone grafts. Orthop Clin North Am 1987;18:179–85.
19. Johnson EE, Urist MR, Finerman, GA. Repair of segmental defects of the tibia with cancellous bone grafts augmented with human bone morphogenetic protein. Clin Orthop 1988; 236:249–57.
20. Johnson EE, Urist MR, Finerman, GA. Bone morphogenetic protein augmentation grafting of resistent femoral nonunions. Clin Orthop 1988;230:257–65.
21. Lane JM, Cornell CN, Werntz JR, Sandhu HS. Clinical applications of biosynthetics. In: Friedlander GE, Goldberg VM, eds. Bone and Cartilage Allografts. Illinois: American Academy of Orthopaedic Surgeons, 1991:279–94.
22. Lane JM, Sandhu HS. Current approaches to experimental bone grafting. Orthop Clin North Am 1987;18:213–25.
23. Lovell TP, Dawson EG, Nilsson OS, Urist MR. Augmentation of spinal fusion with bone morphogenetic protein in dogs. Clin Orthop 1989;243:266–74.
24. Mizutani H, Urist MR. The nature of bone morphogenetic protein (BMP) fractions derived from bovine bone matrix gelatin. Clin Orthop 1982;171:213–23.
25. Nilsson OS, Urist MR, Dawson EG, Schmalzried TP, Finerman GA. Bone repair induced by bone morphogenetic protein in ulnar defects in dogs. J Bone Joint Surg [Br] 1986; 68:635–42.
26. Rosen V, Wozney JM, Wang EA, et al. Purification and molecular cloning of a novel group of BMPs and localization of BMP mRNA in developing bone. Connect Tissue Res 1989; 20:313–9.
27. Sampath TK, Reddi AH. Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci U S A 1981;78: 7599–603.
28. Senn N. On the healing of aseptic cavities by implantation of antiseptic decalcified bone. Am J Med Sci 1889;98:219.
29. Urist MR. Bone formation by autoinduction. Science 1965;150:893–9.
30. Urist MR. Bone morphogenetic protein. In: Habal MB, Reddi AH, eds. Bone Grafts and Bone Substitutes. Philadelphia: WB Saunders, 1992:3–8, 376.
31. Urist MR. The search for and discovery of bone morphogenetic protein (BMP). In: Urist MR, O'Conner BT, Burwell RG, eds. Bone Grafts, Derivatives and Substitutes. London: Butterworth Heinemann, 1994:315–62.
32. Urist MR, DeLange RJ, Finerman GA. Bone cell differentiation and growth factors. Science 1983;220:680–6.
33. Urist MR, Mikulski A, Lietz A. Solubilized and insolubilized bone morphogenetic protein. Proc Natl Acad Sci U S A 1979;76:1828–32.
34. Wang EA, Rosen V, Cordes P, et al. Purification and characterization of other distinct bone-inducing factors. Proc Natl Acad Sci U S A 1988;85:9484–8.
35. Wang EA, Rosen V, D'Alessandro JS, et al. Recombinant human bone morphogenetic protein induces bone formation. Proc Natl Acad Sci U S A 1990;87:2220–4.

36. Wozney J. The bone morphogenetic protein family and osteogenesis. Mol Reprod Dev 1992;32:160–7.
37. Wozney JM, Rosen V, Celeste AJ, et al. Novel regulators of bone formation: Molecular clones and activities. Science 1988;242:1528–34.
38. Yasko AW, Lane JM, Fellinger EJ, Rosen V, Wozney JM, Wang EA. The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). J Bone Joint Surg [Am] 1992;74:659–70.
39. Younger EM, Chapman MW. Morbidity at bone graft donor site. J Orthop Trauma 1989;3:192–5.

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