Osteogenic Protein-1 Delivered by Hydroxyapatite-coated Implants Improves Bone Ingrowth in Extracortical Bone Bridging

Neil Saran MD, FRCSC, Renwen Zhang MD, PhD,
Robert E. Turcotte MD, FRCSC

Received: 27 March 2010 / Accepted: 1 September 2010 / Published online: 28 September 2010
© The Association of Bone and Joint Surgeons® 2010

Abstract

Background Extracortical bone bridging for treatment of massive bone loss can improve stability and longevity of massive endoprostheses. Osteogenic protein-1 (OP-1), when used with allograft bone, reportedly improves extracortical bone bridging and bone ingrowth.

Questions/purposes We asked whether OP-1 delivered by hydroxyapatite (HA) without bone grafting could improve bone ingrowth and bone formation in the context of extracortical bone bridging.

Methods We implanted unilateral segmental femoral diaphyseal replacement prostheses in 18 dogs (three groups

of six dogs). The groups consisted of an HA-coated group augmented with OP-1, an HA-coated group, and a plain porous group. Bone grafting techniques were not used to augment bone formation. The implants were retrieved at 12 weeks for histologic assessment.

Results After removing one specimen owing to a complication, 17 femora were analyzed (six HA-coated augmented with OP-1, five HA-coated, and six plain). We observed better bone ingrowth in the HA-coated OP-1 group than in the plain porous and HA-coated groups, with no difference between the latter two groups. There also was better bone apposition and callus height in the HA-coated OP-1 group than in the plain group but no differences between the HA-coated OP-1 and HA-coated groups or between the HA-coated and plain groups.

Conclusions OP-1 (2.9 mg) delivered by HA-coated segmental replacement prostheses in this canine extracortical bone bridging model revealed improved bone ingrowth over HA-coated implants without OP-1 or plain porous-coated prostheses.

Clinical Relevance OP-1 may be useful as an adjunct to prosthetic reconstruction of massive bone loss without the need for complex bone grafting techniques.

The institution of the authors (NS, RT) has received funding from Stryker Orthopaedics (Mahwah, NJ) for this study. One or more of the authors (RZ) has received payments or benefits from a commercial entity (Stryker Orthopaedics) related to this work. One or more of the authors (RZ) has stock ownership, equity interest, patent/licensing arrangements and/or royalties from Stryker Orthopaedics that could be construed as related to this manuscript.

Each author certifies that his or her institution approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research. This work was performed at the McGill University Health Centre (animal care, surgery, and necropsy including all radiography) and Stryker Orthopaedics (histologic processing).

N. Saran, R. E. Turcotte (✉)
Division of Orthopaedic Surgery, McGill University Health
Centre, Montreal General Hospital, 1650 Cedar Avenue,
Room B5.159.6, Montreal, QC H3G 1A4, Canada
e-mail: robert.turcotte@mhmc.mcgill.ca

N. Saran
e-mail: nsaran77@hotmail.com

R. Zhang
Orthobiologics Group, Stryker Orthopaedics, Mahwah,
NJ, USA

Introduction

Massive endoprostheses are invaluable in dealing with failed total joint arthroplasties or severe bone loss. Although useful reconstructive implants, they are associated with relatively high failure rates [23, 26, 27, 35, 45]. Aseptic loosening remains a primary mode of failure despite advances in the design of these implants [3, 6, 21, 23, 26, 27, 35, 45] with rates ranging from 2.9% to 28.6% at 4 to 10 years [21–23, 26, 27, 35].

The concept of obtaining bone ingrowth at the junction of the body of the implant and the host bone (extracortical bone bridging) came from the observation that heterotopic bone formation, albeit in variable amounts, often occurs at this site after large bony resections [6, 48]. This concept has been explored with attempts to replace large segmental diaphyseal defects with porous-coated surface implants [5]. Ideally one would like to drive and enhance this bone production toward the implant surface such that this new bone binds the recipient implant to improve its stability. Chao et al. showed that extracortical bone bridging has been associated with a low rate of aseptic loosening of 3.6% at 10 years and suggested it as an attractive method to provide durable implant fixation [5]. Furthermore, a finite element analysis suggests that extracortical bone bridging reduces stresses on the stem and cement mantle of such prostheses [7]. Thus, extracortical bone bridging may increase the longevity of such implants [8]. Other benefits of extracortical bone bridging and ingrowth include the possible sealing effect of the fibroosseous sleeve that theoretically prevents wear debris from entering the medullary canal, thereby, decreasing the risk of osteolysis at the bone-cement-implant interfaces [48].

Growth factors such as the TGF- β 1 and - β 2 and bone morphogenetic proteins (BMP)-2 and -7 have enhanced bone ingrowth and bone formation in many animal models [1, 2, 4, 10–13, 25, 28–34, 36–38, 41, 42, 49, 50]. Recombinant human OP-1, also known as BMP-7, is one such growth factor with powerful osteoinductive properties [16] that enhances new bone formation and bone ingrowth [2, 17]. Although one study proposed using autogenous corticocancellous onlay bone grafting at the bone-implant junction for superior bone formation, extracortical bone bridging, bone ingrowth, and soft tissue capsule formation around the prosthesis [47], others have proposed using growth factors with or without bone grafting to improve these processes [17, 37, 40]. A recent report indicates that cortical allograft used in combination with OP-1 in a canine model improves implant biomechanical stability in comparison to using autogenous bone grafting alone [17]. Although OP-1 generally is delivered by a collagen matrix [11, 12, 15, 17], HA, which also is thought to enhance bone ingrowth and osseointegration [9, 14, 20, 39, 44, 46, 51], has been proposed as a carrier for OP-1 [51, 52].

Our primary goal in this study was to assess the amount of bone ingrowth in the adjacent porous-coated region of a segmental replacement prosthesis when OP-1 is delivered by an HA coating. Our secondary goals were to assess the amount of histologic bone formation, histologic bone apposition, and radiographic bone formation when using OP-1 delivered by an HA coating.

Materials and Methods

An experimental canine study was performed with one group being treated with OP-1 delivered by HA, another group being treated with an HA-coated prosthesis, and a third group treated with a prosthesis without the HA coating, to compare bone ingrowth and formation histologically and radiographically to study the effects of OP-1 on these parameters. Approval for the study was obtained from our institutional animal care committee and all guidelines set by our national Council on Animal Care and the University were followed.

A sample size calculation was done to find an absolute difference of 20% depth of bone ingrowth with $\alpha = 0.05$ and $\beta = 0.1$. The expected standard deviation of 13 and effect size of 20% for the calculation were estimated from data on the use of OP-1 in canine acetabular defects [2]. Although biomechanical data for this type of model were not available at the time, a recent study showed a difference of 12% bone ingrowth in this model was associated with a 2.3-fold improvement in torsional stiffness and 2.2-fold maximum torque [17]. The calculation revealed that six animals would be required per group.

Three groups of six skeletally mature mongrel canines weighing 32 to 43 kg (mean, 37 kg) were used. Group one (OPPA) was implanted with an OP-1-augmented Peri-Apatite^{TM} (PA)-coated (Stryker Orthopaedics, Mahwah, NJ, USA) beaded implant. Group two (PAC) was implanted with a PA-coated implant. PA is an HA coating that forms a larger surface area for contact than traditional plasma-sprayed HA coatings [53]. Group three (plain) was implanted with the plain beaded implant.

The prosthesis was developed specifically for the canine diaphyseal segmental replacement model and was used in a previous study [17]. It is a modular cobalt-chromium alloy bitemmed prosthesis with a 30-mm proximal and 40-mm distal stem. The stems are fluted and measure 10 mm in diameter allowing for cement fixation. The two components of the modular implant couple using a Morse taper. The 60-mm segmental body consists of a dual-layered sintered cobalt-chromium alloy bead (diameter, 600–800 μ m) coating (Fig. 1). Twelve of the 18 implants were further coated with PA.

The dogs were anesthetized using 15 to 20 mg of sodium pentathol per kg body weight for induction and 2% to 4% isoflurane with 2 L oxygen per minute for maintenance. The OP-1 (2.9 mg) in 600 μ L buffer solution (provided by Stryker BioTech, Hopkinton, MA, USA) was evenly pipetted onto the porous coat at the beginning of the procedure to allow the PA to absorb the OP-1. Time from application of the OP-1 to the implant until prosthetic implantation was approximately 30 minutes. Control implants were loaded with the same amount of buffer

Fig. 1 A photograph shows the modular bistemmed segmental replacement prosthesis.

solution for the same amount of time before implantation. A lateral approach to the femur was performed followed by a 62-mm extraperiosteal diaphyseal segmental resection using an oscillating saw. Rotational markings were made before the resection to minimize malrotation. The saw blade and bone ends were irrigated continuously to minimize thermal necrosis. The proximal and distal femoral canals were reamed sequentially to 10 mm using blunt tipped rigid reamers. Hemostasis was achieved and final irrigation of the wound was performed at this point before implantation. Vacuum-mixed cement then was injected into proximal and distal femoral canals. The proximal and distal implant segments then were seated, excess cement was removed, and the cement was allowed to cure. The implant then was coupled and the wound was closed in layers ensuring the porous coat of the implant was circumferentially covered by muscle. The skin was closed with a buried running subcuticular Monocryl® suture (Ethicon, Inc, Somerville, NJ, USA). Immediate postoperative radiographs were taken to ensure adequate positioning of the implants.

Cephalexin was continued for 10 days at a dose of 25 to 50 mg per kg body weight per day given twice a day. Pain management included 0.01 to 0.02 mg buprenorphine per kg body weight given intramuscularly every 8 to 10 hours for the first 12 hours after which a 72-hour 75 µg fentanyl transdermal patch was applied. The animals were allowed to weightbear as tolerated postoperatively.

There was one major complication that occurred in one animal in the PAC group. The cement had cured prematurely before insertion of the distal component of the prosthesis. As such, the cemented canal was drilled and reamed. During this process, an anterior perforation occurred and a cement plug was placed and allowed to harden before cementing in the distal component. Postoperative radiographs showed extramedullary placement of the distal part of the stem along with some cement extrusion into the suprapatellar pouch of the knee. Postoperatively, the animal was limping, and therefore, the cement was removed via arthrotomy. The clinical picture improved after the arthrotomy and the animal resumed use of the limb; however, weightbearing was limited on that limb for

Table 1. Radiographic and histomorphometric data

Treatment group	Specimen	Radiographic	Histomorphometric		
		Bone formation (mm ²)	Depth ingrowth (%)	Apposition (µm)	Callus height (µm)
OPPA	1	268	86	17,660	4510
	2	1933	96	7215	1190
	3	126	43	2980	3215
	4	1402	89	11,660	6180
	5	173	77	6330	20,915
	6	1209	89	13,085	4690
PAC	1	139	0	0	250
	2	600	12	7840	4225
	3	145	15	4785	3495
	4	175	0	0	0
	5	162	0	0	740
Plain	1	133	0	840	2060
	2	572	0	0	960
	3	130	12	4125	730
	4	106	0	235	1060
	5	72	0	0	2555
	6	107	0	140	110

OPPA = implanted with an osteogenic protein-1-augmented Peri-Apatite™-coated beaded implant; PAC = implanted with a Peri-Apatite™-coated beaded implant; plain = implanted with the plain beaded implant.

the remaining 12 weeks of the study period. At necropsy, the distal femoral-implant interface was grossly loose, whereas all other specimens appeared stable to manual testing. We excluded this animal for the bone ingrowth analysis as gross motion would prevent bone ingrowth and could artificially give more strength to our OPPA group. At 12 weeks, the animals were euthanized using a lethal dose (90 mg/kg) of intravenous sodium pentobarbital. The femora were harvested, removing all muscle tissue from the femur and implant and leaving behind only bone and fibrous tissues. The specimens were assessed manually to ensure that the unit (proximal femur, prosthesis, and distal femur) was not grossly loose. Radiographic and histologic data were collected for 17 of the 18 animals (Table 1).

High-resolution AP and lateral radiographs were taken using a Faxitron® apparatus (Hewlett-Packard, Boise, ID, USA), which then were digitized (Fig. 2) and analyzed for radiographic bone formation using Bioquant Image Analysis software (Bioquant Image Analysis, Nashville, TN, USA). The area of bone formation seen on the AP and lateral images was captured by the imaging software and these areas were summed for each animal for comparison among the groups.

The proximal and distal ends of the femur 40 mm away from the implant-bone interface were removed to facilitate

Fig. 2A–D The images are examples of radiographs of harvested femora. The (A) AP and (B) lateral radiographs show little bone formation. These (C) AP and (D) lateral radiographs show bridging callus over the posterior aspect of the implant.

storage and the remaining segments were fixed in 70% ethanol, dehydrated, embedded in Spurr plastic, and sectioned to create a 20-mm long proximal and distal segment to enable observation of the junction between the femur and the porous-coated implant segment. One longitudinal section was made for each femur-implant junction. The longitudinal sections were made through the center of the implant axis such that the section would include the area with maximal callus thickness and that the cuts would be perpendicular to the tangential of the implant surface to minimize obliquity through the porous region or the callus. The sections were ground to a final thickness of approximately 40 μm and were stained with Giemsa. The slides were observed under a light microscope (Nikon Eclipse 90i; Nikon Instruments, Inc, Melville, NY, USA) and digital photographs of $\times 10$ magnification were taken for image analysis. Quantitative data were collected with Image-Pro® Plus software (Media Cybernetics, Silver Spring, MD, USA). The depth of the bone ingrowth was expressed as the percentage of the deepest point of the bone inside the porous coating to the whole thickness of the porous coating. Bone apposition was expressed as the total length of the surface of the implant that has bone attachment of 50% or more of its length. The callus height was measured on each image as the maximum thickness of callus from the implant surface. All histologic measurements were performed by one author (RZ) who was blinded to the treatment groups.

We determined differences in bone ingrowth between the three groups using the Kruskal-Wallis rank-sum test with a p value set to 0.05 for significance. A Wilcoxon rank-sum test was used for multiple comparisons testing

within the groups and after applying a Bonferroni correction for multiple comparisons, a p value of 0.016 was considered significant. Similarly, the Kruskal-Wallis rank-sum test was used to determine differences in histologic callus height and bone apposition, and radiographic bone formation, callus height, and bone apposition between the three groups and the Wilcoxon rank-sum test was used for multiple comparisons testing within the three groups for each of these parameters with a p value of 0.016 set for significance after a Bonferroni correction was applied for multiple comparisons. All analyses were performed using S-Plus® 8.0 (Insightful Corp, Seattle, WA, USA).

Results

Histologic data revealed differences ($p = 0.002$) in bone ingrowth among the three treatments (Table 2): the OP-1 treatment was associated with greater bone ingrowth than PAC ($p = 0.008$) and plain ($p = 0.004$), whereas there was no difference between PAC and plain ($p = 0.35$) (Table 3).

We observed differences ($p = 0.017$) in bone apposition among the three groups. The OP-1 treatment amounted in greater apposition than the plain group ($p = 0.008$) but not the PAC group ($p = 0.05$). There was no difference in bone apposition between the PAC and plain groups ($p = 1$). There was an overall difference ($p = 0.025$) in callus height between the three groups. However, although there was a difference between the OP-1 and plain groups ($p = 0.009$), we found none between the OP-1 and PAC groups ($p = 0.05$) or the PAC and plain groups ($p = 1$).

Table 2. Bone formation, depth of ingrowth, apposition, and callus height data

Variable	OPPA	PAC	Plain	Kruskal-Wallis rank-sum p value
Bone formation (mm ²)	852 ± 765 (738.5, 126–1933)	244 ± 182 (162, 139–600)	186 ± 190 (118.5, 72–572)	0.023
Depth of ingrowth (%)	80.2 ± 19.4 (87.5, 43–96)	5.5 ± 7.6 (0, 0–15)	2.0 ± 4.9 (0, 0–12)	0.002
Apposition (µm)	9822 ± 5315 (9438, 2981–17,662)	2525 ± 3622 (0, 0–4125)	890 ± 1615 (187.5, 0–7839)	0.017
Callus height (µm)	6783 ± 7121 (4600, 1190–20,914)	1742 ± 1968 (740, 0–4224)	1246 ± 900 (1010, 111–2553)	0.025

Values are expressed as mean ± SD, with median and range in parentheses; OPPA = implanted with an osteogenic protein-1-augmented Peri-Apatite™-coated beaded implant; PAC = implanted with a Peri-Apatite™-coated beaded implant; plain = implanted with the plain beaded implant.

Table 3. Statistical summary of multiple comparisons

Type of data	Variable	p Value		
		OPPA versus PAC	OPPA versus plain	PAC versus plain
Radiographic	Bone formation	0.13	0.03	0.05
Histomorphometric	Depth of ingrowth	0.008	0.004	0.35
	Apposition	0.05	0.008	1
	Callus height	0.05	0.009	1

OPPA = implanted with an osteogenic protein-1-augmented Peri-Apatite™-coated beaded implant; PAC = implanted with a Peri-Apatite™-coated beaded implant; plain = implanted with the plain beaded implant.

We observed no difference in radiographic bone formation between the three groups based on the multiple comparisons testing (Table 3).

Thin section histologic analysis revealed that new bone formation occurred mostly from the periosteum adjacent to the cut ends of the bone, the PA coating of the beaded surface was mostly absorbed by the time the specimens were harvested, the OPPA group seemed to show more bone ingrowth and bone formation than the other two groups, and bone formation around the shoulder and porous-coated region did not always equate to bone ingrowth into the porous-coated region of the implant (Fig. 3). Another important finding was, when bone bridging occurred, it was contiguous with the adjacent cortical bone and the porous coat of the prosthesis.

Discussion

Achieving extracortical bone bridging and bone ingrowth should increase the longevity of bone and joint reconstructive implants. It is postulated OP-1 when used with PA may improve bone bridging and bone ingrowth. The primary goal of this study was to assess the amount of bone ingrowth into the adjacent porous-coated region of a

segmental replacement prosthesis when OP-1 is delivered by an HA coating. Our secondary goals were to assess the amount of histologic bone formation, histologic bone apposition, and radiographic bone formation when using OP-1 delivered by an HA coating.

There are numerous limitations in this study. First, the number of animals used is small. As such, not only was it necessary to use nonparametric statistical tests, but also it increases the probability of making a Type II error. Second, the thin sections were made in the longitudinal rather than the transverse plane. In doing so, the sections were taken from the area believed to reveal the best bone formation and enabled comparison of the best-case scenario of each group. Furthermore, the longitudinal sections enabled us to show the ingrowth into the porous coat was contiguous with the adjacent bone. Third, ours is a short-term study and it is difficult to speculate on how the newly formed extracortical bone and bone ingrowth will remodel in the long term. The biomechanical properties of the cobalt-chromium alloy prosthesis used in this study are unknown, and implant stiffness may play an important role in the long-term remodeling of extracortical bone. Although we do not have long-term data, bone ingrowth by extracortical bone bridging decreases bone-implant stresses [7] and acts in a load-sharing capacity [5] which should prevent resorption of the newly formed bone and may in fact enhance its integrity. Fourth, our study groups did not include a group using OP-1 without the PA-coated prosthesis. Our reasoning for this is that the PA-coating enables some form of delivery of the OP-1 while fully understanding that the release kinetics and local concentrations of OP-1 using such a delivery system are not fully known. However, it is known that PA-coated discs absorb OP-1 in a linear fashion when applied in concentrations of up to 5 mg/mL, 75% to 80% of the adsorbed OP-1 is released within the first hour of implantation in such a delivery system, and that 92% of the OP-1 is released by 3 days of implantation [52]. Based on these data [52], we think PA is a justifiable carrier for OP-1 although additional studies on release kinetics would help to better define such a role. Fifth, only a large single dose was used in this study as has

Fig. 3A–F Longitudinal histologic sections of specimens show various degrees of bone ingrowth and bone formation. (A) A PA-coated implant shows mainly fibrous tissue ongrowth with little new bone formation and ingrowth (Stain, Giesma; original magnification, $\times 40$). (B) The same specimen at a higher magnification is shown (Original magnification, $\times 100$). (C) A PA-coated implant treated with OP-1 shows new bone formation from the adjacent periosteum (black arrow) with good bone ingrowth. Continuity of the newly formed bone and the cortex also can be seen (Stain, Giesma; original magnification, $\times 40$). (D) Another PA-coated implant treated with OP-1 specimen at higher magnification shows bone ingrowth (Stain, Giesma; original magnification, $\times 100$). (E) A PA-coated implant shows moderate bone formation from the adjacent cortex but minimal bone ingrowth (Stain, Giesma; original magnification, $\times 40$). (F) The same specimen at higher magnification shows fibrous tissue ingrowth but no bone ingrowth (Original magnification, $\times 100$).

been the case in other similar very large defect canine models [11, 15, 17, 38]. Although our study suggests that OP-1 delivered by PA potentiates bone ingrowth in extracortical bone bridging, determination of optimal dosing requires further work. Finally, the use of OP-1 may not be indicated for tumor cases and should likely be reserved for cases of massive bone loss secondary to nontumor-related reasons in which extracortical bone bridging may be desirable.

The findings of superior bone ingrowth when OP-1 was used with PA-coated porous prostheses in diaphyseal segmental defects in this canine model are similar to those previously reported. In a canine model, Barrack et al. [2] found that acetabular defects filled with OP-1 resulted in

better ingrowth of acetabular components. Zhang et al. [51] found that bone ingrowth and bone healing were superior in a rabbit gap model. Fukuroku et al. [17] found that although mechanical stability was improved when OP-1 was used with allograft bone to achieve extracortical bone bridging, bone ingrowth was not superior to autogenous bone graft without the use of OP-1. Although all of these other models have evaluated ingrowth, they have used some form of osteoconductive surrounding that could in some way support bone formation. Pluhar et al. [37] found recombinant human BMP-2 without the use of bone graft can improve bone formation in the extracortical bone-bridging model at the junction of the implant and bone; however, the quantification of bone formation was purely

radiographic and ingrowth was not assessed. In a human implant retrieval study, Tanzer et al. [43] found radiographic ingrowth does not correlate to histologic ingrowth. Our study showed histologically that bone ingrowth can occur without the use of bone graft when OP-1 is delivered by PA. Whether bone grafting can improve extracortical bone bridging or bone ingrowth when used as an adjunct to OP-1 remains an unanswered question that warrants further evaluation.

We found that although there was no difference in the amount of radiographic bone formation between the three groups, the histologically-measured callus height over the immediately adjacent porous region was greater for the OP-1 loaded group as compared with the plain group but not between the OP-1 loaded and PA only groups or between the PA only and plain groups. A number of studies demonstrate OP-1, when used in gap or defect models, improves bone formation [11, 12, 15, 34]. Fukuroku et al. [17] found no differences in bone formation between OP-1 with allograft and autograft alone and suggested that perhaps the small sample size of seven per group was a possible reason for the lack of statistical superiority.

Although the OP-1 loaded group had better histologic bone apposition than the plain group, there was no difference between the OP-1 loaded and the PA only groups or between the PA only and plain groups. In an intraosseous gap model, Lind et al. [34] studied bone ongrowth with respect to HA coating, OP-1 and a collagen matrix. They found HA groups and OP-1 groups improved bone ongrowth and although they observed no difference in the amounts of bone ongrowth between these two groups, the OP-1 device was associated with improved mechanical fixation of uncoated and HA-coated implants. We did not find a difference between the PA only and the plain groups in our study.

In our study, the sole addition of PA to the basic implant did not appear to enhance bone formation, apposition, or ingrowth. There has been debate regarding the exact mechanism by which osseointegration is improved by HA. Although studies have postulated the biochemical similarity of HA to bone may explain its osteoconductive properties [18, 19], it has been suggested it is likely the microsurface topography of HA rather than its chemistry that promotes osseointegration [24]. Regardless of the mechanism of action, many studies show improved bone ingrowth, mechanical strength, and osseointegration with its use [9, 14, 20, 39, 46, 51]. In our study, there was no difference in extracortical bone bridging or bone ingrowth between the PA and plain groups. The expectation was that the PA may reveal slightly better new bone formation than the plain porous-coated prosthesis; however, without any bone

grafting, perhaps there were not enough osteoinductive and osteogenic factors around to stimulate bone formation. Again, this suggests the importance of using OP-1 or autogenous bone graft to improve new bone formation and osseointegration.

The qualitative assessment of the histology showed that the new bone appears to form from the adjacent periosteum. This finding is supported by Nilsson et al. [36], who also reported that BMP-induced new bone formation arises from adjacent periosteum. Fukuroku et al. [17] preserved the periosteum over the reconstructed segment in their model and commented that preserved periosteum could have important implications on extracortical bone bridging and ingrowth. We too think that preservation of adjacent periosteum such that it can be laid onto the porous-coated region of the implant is an important feature that could potentiate bone formation and bone ingrowth.

A high dose of OP-1 (2.9 mg) was used in this study. Various dosages of OP-1 have been used in different animal models. Low doses (6.25 µg) enhance the likelihood of union in a rabbit ulna nonunion model [12], and small gap (3 mm) models in canines show improved bone formation and ingrowth with doses of 300 to 325 µg [25, 34]; whereas, large defect (> 20 mm) models in large animals (primates and canines) have tended to use larger OP-1 doses (1–3.5 mg) to show improved bone formation, biomechanical strength, and bone ingrowth [11, 15, 17, 38]. A dose comparison study of the use of 1.75 mg versus 3.5 mg of OP-1 when delivered by a carboxymethylcellulose collagen combination carrier in a 25-mm canine ulnar defect model showed greater early bone formation in the larger dose group; however, final radiographic and histologic healing and mechanical strength testing showed no difference between the two doses suggesting that a lesser dose could be used [13]. Comparison studies for dose effects on bone ingrowth and bone formation in very large defects (60 mm in this case) do not exist and determination of optimal dosing requires further investigation.

We showed OP-1 (2.9 mg) when used in combination with PA-coated prostheses in a canine segmental defect model can improve bone ingrowth in the extracortical bone bridging model. Although the PA-coated implants when used in isolation (without OP-1) did not appear to enhance extracortical bridging, our results and those of others [51, 52] suggest they may be useful in the delivery of biologic growth factors such as OP-1. Additional work is required to better define the absorptive and elution characteristics of PA and determine the importance of autogenous bone grafting when OP-1 is used to enhance extracortical bone bridging and bone ingrowth.

Acknowledgments We thank Thomas Bauer MD, for help with the histopathologic analysis and Marie-Eve Robitaille from our institutional animal laboratory for help with our efforts and in caring for the animals during the research period.

References

1. Aebli N, Stich H, Schawalter P, Theis JC, Krebs J. Effects of bone morphogenetic protein-2 and hyaluronic acid on the osseointegration of hydroxyapatite-coated implants: an experimental study in sheep. *J Biomed Mater Res A*. 2005;73:295–302.
2. Barrack RL, Cook SD, Patron LP, Salkeld SL, Szuszczewicz E, Whitecloud TS III. Induction of bone ingrowth from acetabular defects to a porous surface with OP-1. *Clin Orthop Relat Res*. 2003;417:41–49.
3. Blunn GW, Briggs TW, Cannon SR, Walker PS, Unwin PS, Culligan S, Cobb JP. Cementless fixation for primary segmental bone tumor endoprostheses. *Clin Orthop Relat Res*. 2000;372:223–230.
4. Bragdon CR, Doherty AM, Rubash HE, Jasty M, Li XJ, Seeherman H, Harris WH. The efficacy of BMP-2 to induce bone ingrowth in a total hip replacement model. *Clin Orthop Relat Res*. 2003;417:50–61.
5. Chao EY, Fuchs B, Rowland CM, Ilstrup DM, Pritchard DJ, Sim FH. Long-term results of segmental prosthesis fixation by extracortical bone-bridging and ingrowth. *J Bone Joint Surg Am*. 2004;86:948–955.
6. Chao EY, Sim FH. Modular prosthetic system for segmental bone and joint replacement after tumor resection. *Orthopedics*. 1985;8:641–651.
7. Chao EY, Sim FH. Composite fixation of segmental bone/joint defect replacement (SDR) prostheses: biological and biomechanical justifications. *Chir Organi Mov*. 1990;75(1 suppl):171–173.
8. Chao EY, Sim FH. Composite fixation of salvage prostheses for the hip and knee. *Clin Orthop Relat Res*. 1992;276:91–101.
9. Coathup MJ, Blunn GW, Flynn N, Williams C, Thomas NP. A comparison of bone remodelling around hydroxyapatite-coated, porous-coated and grit-blasted hip replacements retrieved at post-mortem. *J Bone Joint Surg Br*. 2001;83:118–123.
10. Cole BJ, Bostrom MP, Pritchard TL, Sumner DR, Tomin E, Lane JM, Weiland AJ. Use of bone morphogenetic protein 2 on ectopic porous coated implants in the rat. *Clin Orthop Relat Res*. 1997;345:219–228.
11. Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC. Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone segmental defect model. *Clin Orthop Relat Res*. 1994;301:302–312.
12. Cook SD, Baffes GC, Wolfe MW, Sampath TK, Rueger DC, Whitecloud TS 3rd. The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. *J Bone Joint Surg Am*. 1994;76:827–838.
13. Cook SD, Salkeld SL, Patron LP. Bone defect healing with an osteogenic protein-1 device combined with carboxymethylcellulose. *J Biomed Mater Res B Appl Biomater*. 2005;75:137–145.
14. Cook SD, Thomas KA, Dalton JE, Volkman TK, Whitecloud TS 3rd, Kay JF. Hydroxylapatite coating of porous implants improves bone ingrowth and interface attachment strength. *J Biomed Mater Res*. 1992;26:989–1001.
15. Cook SD, Wolfe MW, Salkeld SL, Rueger DC. Effect of recombinant human osteogenic protein-1 on healing of segmental defects in non-human primates. *J Bone Joint Surg Am*. 1995;77:734–750.
16. Croteau S, Rauch F, Silvestri A, Hamdy RC. Bone morphogenetic proteins in orthopedics: from basic science to clinical practice. *Orthopedics*. 1999;22:686–695; quiz 696–697.
17. Fukuroku J, Inoue N, Rafiee B, Sim FH, Frassica FJ, Chao EY. Extracortical bone-bridging fixation with use of cortical allograft and recombinant human osteogenic protein-1. *J Bone Joint Surg Am*. 2007;89:1486–1496.
18. Geesink RG. Hydroxyapatite-coated total hip prostheses: two-year clinical and roentgenographic results of 100 cases. *Clin Orthop Relat Res*. 1990;261:39–58.
19. Geesink RG, de Groot K, Klein CP. Chemical implant fixation using hydroxyl-apatite coatings: the development of a human total hip prosthesis for chemical fixation to bone using hydroxyl-apatite coatings on titanium substrates. *Clin Orthop Relat Res*. 1987;225:147–170.
20. Geesink RG, de Groot K, Klein CP. Bonding of bone to apatite-coated implants. *J Bone Joint Surg Br*. 1988;70:17–22.
21. Gosheger G, Gebert C, Ahrens H, Streithuerger A, Winkelmann W, Hardes J. Endoprosthetic reconstruction in 250 patients with sarcoma. *Clin Orthop Relat Res*. 2006;450:164–171.
22. Griffin AM, Parsons JA, Davis AM, Bell RS, Wunder JS. Uncemented tumor endoprostheses at the knee: root causes of failure. *Clin Orthop Relat Res*. 2005;438:71–79.
23. Guo W, Ji T, Yang R, Tang X, Yang Y. Endoprosthetic replacement for primary tumours around the knee: experience from Peking University. *J Bone Joint Surg Br*. 2008;90:1084–1089.
24. Hacking SA, Tanzer M, Harvey EJ, Krygier JJ, Bobyn JD. Relative contributions of chemistry and topography to the osseointegration of hydroxyapatite coatings. *Clin Orthop Relat Res*. 2002;405:24–38.
25. Jensen TB, Overgaard S, Lind M, Rahbek O, Bunger C, Soballe K. Osteogenic protein 1 device increases bone formation and bone graft resorption around cementless implants. *Acta Orthop Scand*. 2002;73:31–39.
26. Jeys LM, Kulkarni A, Grimer RJ, Carter SR, Tillman RM, Abudu A. Endoprosthetic reconstruction for the treatment of musculoskeletal tumors of the appendicular skeleton and pelvis. *J Bone Joint Surg Am*. 2008;90:1265–1271.
27. Kawai A, Muschler GF, Lane JM, Otis JC, Healey JH. Prosthetic knee replacement after resection of a malignant tumor of the distal part of the femur: medium to long-term results. *J Bone Joint Surg Am*. 1998;80:636–647.
28. Kirker-Head CA, Gerhart TN, Armstrong R, Schelling SH, Carmel LA. Healing bone using recombinant human bone morphogenetic protein 2 and copolymer. *Clin Orthop Relat Res*. 1998;349:205–217.
29. Kirker-Head CA, Gerhart TN, Schelling SH, Hennig GE, Wang E, Holtrop ME. Long-term healing of bone using recombinant human bone morphogenetic protein 2. *Clin Orthop Relat Res*. 1995;318:222–230.
30. Lind M, Overgaard S, Jensen TB, Song Y, Goodman SB, Bunger C, Soballe K. Effect of osteogenic protein 1/collagen composite combined with impacted allograft around hydroxyapatite-coated titanium alloy implants is moderate. *J Biomed Mater Res*. 2001;55:89–95.
31. Lind M, Overgaard S, Nguyen T, Ongpipattanakul B, Bunger C, Soballe K. Transforming growth factor-beta stimulates bone ongrowth: hydroxyapatite-coated implants studied in dogs. *Acta Orthop Scand*. 1996;67:611–616.
32. Lind M, Overgaard S, Ongpipattanakul B, Nguyen T, Bunger C, Soballe K. Transforming growth factor-beta 1 stimulates bone ongrowth to weight-loaded tricalcium phosphate coated implants: an experimental study in dogs. *J Bone Joint Surg Br*. 1996;78:377–382.

33. Lind M, Overgaard S, Soballe K, Nguyen T, Ongpipattanakul B, Bunger C. Transforming growth factor-beta 1 enhances bone healing to unloaded tricalcium phosphate coated implants: an experimental study in dogs. *J Orthop Res*. 1996;14:343–350.
34. Lind M, Overgaard S, Song Y, Goodman SB, Bunger C, Soballe K. Osteogenic protein 1 device stimulates bone healing to hydroxyapatite-coated and titanium implants. *J Arthroplasty*. 2000;15:339–346.
35. Malawer MM, Chou LB. Prosthetic survival and clinical results with use of large-segment replacements in the treatment of high-grade bone sarcomas. *J Bone Joint Surg Am*. 1995;77:1154–1165.
36. 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;68:635–642.
37. Pluhar GE, Manley PA, Heiner JP, Vanderby R Jr, Seeherman HJ, Markel MD. The effect of recombinant human bone morphogenetic protein-2 on femoral reconstruction with an intercalary allograft in a dog model. *J Orthop Res*. 2001;19:308–317.
38. Salkeld SL, Patron LP, Barrack RL, Cook SD. The effect of osteogenic protein-1 on the healing of segmental bone defects treated with autograft or allograft bone. *J Bone Joint Surg Am*. 2001;83:803–816.
39. Soballe K, Hansen ES, Brockstedt-Rasmussen H, Hjordtal VE, Juhl GI, Pedersen CM, Hvid I, Bunger C. Gap healing enhanced by hydroxyapatite coating in dogs. *Clin Orthop Relat Res*. 1991;272:300–307.
40. Stevenson S, Cunningham N, Toth J, Davy D, Reddi AH. The effect of osteogenin (a bone morphogenetic protein) on the formation of bone in orthotopic segmental defects in rats. *J Bone Joint Surg Am*. 1994;76:1676–1687.
41. Sumner DR, Turner TM, Urban RM, Leven RM, Hawkins M, Nichols EH, McPherson JM, Galante JO. Locally delivered rhTGF-beta2 enhances bone ingrowth and bone regeneration at local and remote sites of skeletal injury. *J Orthop Res*. 2001;19:85–94.
42. Sumner DR, Turner TM, Urban RM, Turek T, Seeherman H, Wozney JM. Locally delivered rhBMP-2 enhances bone ingrowth and gap healing in a canine model. *J Orthop Res*. 2004;22:58–65.
43. Tanzer M, Turcotte R, Harvey E, Bobyn JD. Extracortical bone bridging in tumor endoprostheses: radiographic and histologic analysis. *J Bone Joint Surg Am*. 2003;85:2365–2370.
44. Tonino A, Oosterbos C, Rahmy A, Therin M, Doyle C. Hydroxyapatite-coated acetabular components: histological and histomorphometric analysis of six cups retrieved at autopsy between three and seven years after successful implantation. *J Bone Joint Surg Am*. 2001;83:817–825.
45. Torbert JT, Fox EJ, Hosalkar HS, Ogilvie CM, Lackman RD. Endoprosthetic reconstructions: results of long-term followup of 139 patients. *Clin Orthop Relat Res*. 2005;438:51–59.
46. Tsukeoka T, Suzuki M, Ohtsuki C, Tsuneizumi Y, Miyagi J, Sugino A, Inoue T, Michihiro R, Moriya H. Enhanced fixation of implants by bone ingrowth to titanium fiber mesh: effect of incorporation of hydroxyapatite powder. *J Biomed Mater Res B Appl Biomater*. 2005;75:168–176.
47. Virolainen P, Inoue N, Nagao M, Ohnishi I, Frassica F, Chao EY. Autogenous onlay grafting for enhancement of extracortical tissue formation over porous-coated segmental replacement prostheses. *J Bone Joint Surg Am*. 1999;81:493–499.
48. Ward WG, Johnston KS, Dorey FJ, Eckardt JJ. Extramedullary porous coating to prevent diaphyseal osteolysis and radiolucent lines around proximal tibial replacements: a preliminary report. *J Bone Joint Surg Am*. 1993;75:976–987.
49. Yasko AW, Lane JM, Fellingner EJ, Rosen V, Wozney JM, Wang EA. The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2): a radiographic, histological, and biomechanical study in rats. *J Bone Joint Surg Am*. 1992;74:659–670.
50. Zegzula HD, Buck DC, Brekke J, Wozney JM, Hollinger JO. Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). *J Bone Joint Surg Am*. 1997;79:1778–1790.
51. Zhang R, An Y, Toth CA, Draughn RA, Dimaano NM, Hawkins MV. Osteogenic protein-1 enhances osseointegration of titanium implants coated with peri-apatite in rabbit femoral defect. *J Biomed Mater Res B Appl Biomater*. 2004;71:408–413.
52. Zhang R, Xu D, Landeryou T, Toth C, Dimaano N, Berry J, Evans J, Hawkins M. Ectopic bone formation using osteogenic protein-1 carried by a solution precipitated hydroxyapatite. *J Biomed Mater Res A*. 2004;71:412–418.
53. Zitelli JP, Higham P. A novel method for solution deposition of hydroxyapatite on to three dimensionally porous metallic surfaces: Peri-Apatite HA. *Mat Res Soc Symp Proc*. 2000;599:117–128.