Osteogenic protein 1 device increases bone formation and bone graft resorption around cementless implants

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ABSTRACT – In each femoral condyle of 8 Labrador dogs, a non weight-bearing hydroxyapatite-coated implant was inserted surrounded by a 3 mm gap. Each gap was filled with bone allograft or ProOsteon with or without OP-1 delivered in a bovine collagen type I carrier (OP-1 device). 300 μg OP-1 was used in the 0.75 cc gap surrounding the implant. After 3 weeks, the OP-1 device enhanced implant fixation by 800% ($p < 0.05$) in the ProOsteon group, but OP-1 had no significant effect on implant fixation in the allograft group. By adding the OP-1 device, the volume fraction of woven bone close to the implant increased from 12% to 20% ($p < 0.05$) in the bone allograft group and from 6% to 25% ($p < 0.05$) in the ProOsteon group. The volume fraction of bone allograft decreased from 29% to 9% ($p < 0.05$) in the OP-1 treated group versus 33% to 30% in the allograft group not treated with OP-1. No resorption of ProOsteon was found. In conclusion, OP-1 accelerates resorption of bone allograft and enhances new bone formation around cementless implants grafted with bone allograft or semisynthetic hydroxyapatite bone substitute. Our findings do not support the use of ProOsteon alone around cementless implants.

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Bone grafting procedures are often necessary in revision of hips (Rubash et al. 1998). Autograft is the golden standard as graft material, but sufficient amounts are hard to harvest in major hip revision surgery. Morselized fresh frozen bone allograft (FFBA) from femoral heads, packed in the femoral canal or acetabulum, has been used successfully to treat deficient bone (McDonald et al. 1988, Schreurs et al. 1994, Hubble and Smith 1996, Slooff et al. 1996, Benson et al. 2000). However, bone allograft involves the risk of transmitting diseases (Asselmeier et al. 1993, Salzman et al. 1993, Sutherland et al. 1997), adverse immunological response (Salzman et al. 1993) and the supply is limited (Bos et al. 1983, Goldberg et al. 1984, Goldberg et al. 1985, Sutherland et al. 1997). Bone substitutes, such as tricalcium phosphates, calcium carbonates and hydroxyapatites have been marketed as substitutes for bone allograft. Such substitutes are bone conductors with no osteoinductive ability (el Deeb et al. 1989). One experimental study showed that hydroxyapatite/tricalcium phosphate granules are not as good as a gap-filling material autograft around endoprostheses (Turner et al. 1993), although HA granules have been used clinically in revision surgery (Oonishi et al. 1997). Osteointegration of bone substitutes can be improved by adding osteoinducing proteins (el Deeb et al. 1989, Ripamonti et al.1992, Ono et al. 1996). Recombinant human osteogenic protein-1 (rhOP-1) has previously been mixed with grafting materials, but the results have varied (Salkeld et al. 1997, Lind et al. 1998, Søballe and Bechtold 1999).

In this study, we compared the effects of porous semisynthetic hydroxyapatite granules (ProOsteon) with morselized bone allograft on implant fixation and gap-healing in an experimental implant model. We also evaluated the influence of OP-1 on osteointegration of bone allograft and ProOsteon.

Material and methods

Design

We used 8 Labrador dogs aged 14 months having an average weight of 27.5 (25–30) kg. 1 additional dog served as a donor of bone allograft. The protocol was accepted by the Danish Committee for Animal Research and all handling of animals was done according to Danish laws for research. The observation time was 3 weeks. 4 gaps were created in the femoral condyles in the trabecular bone. Each gap was block randomized to one of the following treatment groups: group 1) allograft; group 2) ProOsteon; group 3) allograft + OP-1 device; and group 4) ProOsteon + OP-1 device. All grafts were standardized for weight. The amount of allograft or ProOsteon was reduced when it was combined with the OP-1 device. To compare volume fractions of grafting materials after 3 weeks to those at the time of implantation, 8 control implants from each treatment group were inserted into cadaver bone using the same materials as in the in vivo experiment. The control implants and surrounding bone were cut en bloc and prepared as described below for histomorphometry.

Grafting material

Bone allograft. The proximal humerus, proximal and distal femur, harvested from a dog not included in the study, were stored in two glass containers at –80 °C. After 2 weeks they were thawed and soft tissue and cartilage were dissected. Using a standard bone mill (Biomet Inc, Warsaw, IN, USA), the bone was milled into a homogeneous substance in an operating room with laminar air flow. Finally, the graft was separated into weighed portions, packed in sterile containers and stored at –80 °C. All procedures were carried out under aseptic conditions and bacterial cultures were taken.

ProOsteon. ProOsteon 200 (Interpore, Irvine, USA) is a commercial corraline porous hydroxyapatite bone substitute having an average porous diameter of 200 μm. ProOsteon 200 was delivered as granules with a diameter of 425–1000 μm. Before operation, it was weighed into portions and autoclaved.

OP-1 device. OP-1 (BMP-7) was delivered in a device with 2.5 mg recombinant human OP-1 in 1 gram bovine type I collagen (Stryker Biotech). The dose of OP-1 was 300 μg OP-1 in a 120 mg collagen carrier.

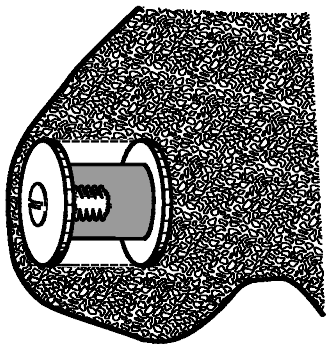


Figure 1. The implant was centered in an overreamed canal surrounded by a 3 mm gap which was grafted.

Implants

Porous HA-coated titanium alloy (Ti-6A1-4V) implants manufactured by Biomet Inc. (Warsaw, IN, USA) were used. The implants were cylindrical in shape, had a length of 10 mm and a final diameter of 5 mm. according to the manufacturer, their mean pore diameter was 480 μm and porosity 44% before the HA coating. A 50 μm HA coating (Ca/P ratio 1.67) was plasma-sprayed by Bio-Interfaces Inc. (San Diego, CA, USA). The surface roughness (Ra) of HA-coated implants was 41 μm, measured as the distance from the mean line to the valley and peak points. Implants were sterilized by gamma irradiation. A standardized 3 mm gap was obtained with a footplate and washer (Figure 1).

Surgery

Anesthesia was induced by intravenous Brietal (10 mg/kg) and maintained by halothane. We inserted unloaded implants into the medial and lateral condyles in both knees, as described elsewhere (Søballe et al. 1992c). A cylindrical hole of 11 mm was hand-drilled and cleaned with saline. The implant was inserted, leaving a 3 mm gap (0.75 cc) between the implant surface and surrounding bone. The gap was filled in accordance with the treatment groups described. Before and after each operation, 1g ampicillin was given . After methohexital sedation, the dogs were killed with an overdose of KCl.

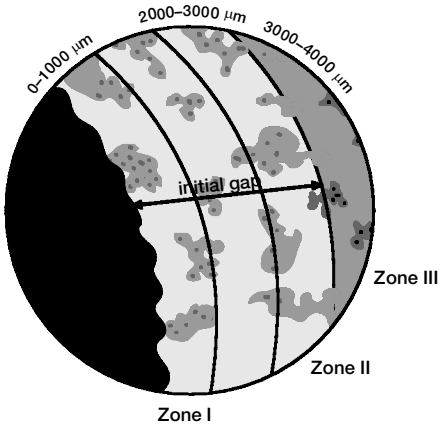


Figure 2. The volume fraction of new bone formation, grafting material and unmineralized tissue were quantified in two zones well defined from the implant surface: Zone 1: 0–1000 μm, zone 2: 2000–3000 μm. The volume fraction of bone was quantified in zone 3, 3000–4000 μm from the implant surface.

Preparation of tissue samples

The distal femurs were harvested and stored at –20 °C. We made sections at a right angle to the axis of each implant on a water-cooled diamond band saw (Exact, Apparatebau, Norderstedt, Germany). The most superficial 5 mm were prepared for histomorphometry, the lower 3.5 mm were stored at –20 °C and used for mechanical testing.

Histomorphometry

The specimens were dehydrated in 70%–100% ethanol containing 0.4% basic fucsin and then embedded in methylmethacrylate (Technovit 7200 VLC, Exact, Apparatebau, Norderstedt, Germany). 4 sections 25 μm thick were cut perpendicular to the long axis of the implant with a microtome (Leiden, Holland) and surface-stained with 2% light green for 2 minutes (Gotfredsen et al. 1989). Bone allograft and woven bone stained green, but could be distinguished by differences in appearance and structure in polarized light (Figure 3)—i.e., allograft had empty lacunae, ProOsteon stained brown (Figure 4) and collagen carrier from the OP-1 device reddish.

The microscope field was transmitted to a monitor. Histomorphometry was done using a software program (CAST-Grid, Olympus, Denmark), which made it possible to specify grids with lines and points. Grids were superimposed on the microscopic field of the monitor. We evaluated bone ongrowth with the linear intercept method. About 250 interceptions on the surface of each implant were counted and bone ongrowth was calculated as bone coverage of the implant surface, as a percentage of the total surface area. Volume fractions of woven bone, grafting material and unmineralized tissue in the gap were estimated 0–1 mm (zone 1) and 2–3 mm (zone 2), respectively, from the implant surface (Figure 2). 275 points were counted at a 100X magnification and an additional 420 points were counted in a 1 mm zone outside the border of the drill-hole (zone 3) and the volume fraction of bone determined. Resorption of the grafting material was calculated as the difference in volume fraction at time zero and after 3 weeks. All specimens were blinded pending analysis of the data.

Mechanical testing

The push-out test was done with an Instron Universal test machine (Instron Ltd. High Wycombe, U.K.). The specimen was placed on a metal support jig and the implant centered over a 7 mm circular opening. A preload of 2 N defined the contact position for starting the test. The implant was displaced at a velocity of 5 mm/minute and load-deformation curves were taken on an x–y recorder (PM 8043;Phillips, Eindhoven, Holland). Ultimate shear strength (σ_u) was determined from the maximal force (F) and calculated as $\sigma_u = F/\pi DL$, where D is the diameter and L the length of the implant. Apparent shear stiffness was obtained from the slope of the straight-line part of the load-displacement curve and calculated as $E = (\delta F/\pi DL)/\delta L$. Energy absorption was calculated from the area beneath the curve until failure.

Statistics

All data are presented as median values and range in brackets. After using the Kruskal-Wallis one-way analysis of variance on ranks, groups were compared pairwise using the Student-Newman-Keuls test. Resorption of the bone allograft or the ProOsteon rate was not compared because they are regarded as resorbable or not resorbable, respectively. P-values less than 0.05 were considered significant.

Table 1. Push-out values. Median (range)

Group	Ultimate shear strength (MPa)	Energy abs. (J/m^2)	Apparent stiffness (MPa/ mm)
Allograft	2.30 (0.49–4.75)	330 (144–719)	16.1 (13.0–26.0)
ProOsteon	0.39 (0–0.58) [a]	83 (0–357) [a]	1.4 (0–2.1) [a]
Allograft+OP-1	1.91 (0.38–4.83)	346 (61–705)	13.1 (7.5–35.9)
ProOsteon+OP-1	2.56 (0.82–5.67)	382 (205–719)	25.9 (4.3–50.3)

[a] $p < 0.05$, compared to the other 3 groups

Results

Surgery

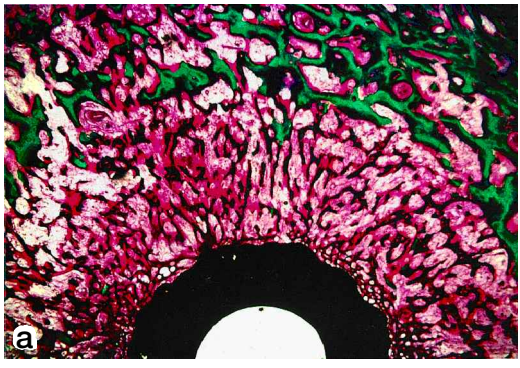
2 dogs were excluded after 2 weeks due to rehabilitation problems. Postmortem testing of the knees showed instability, probably due to release of the collateral ligaments. 6 dogs were killed after 3 weeks. None of the implants showed signs of infections and all cultures from the bone allografts were negative.

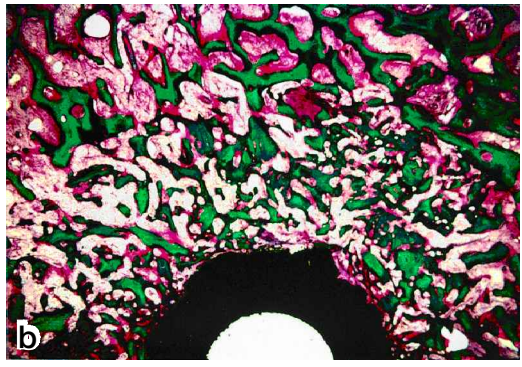
Mechanical tests

Bone allograft showed significantly better fixation than ProOsteon without OP-1 device. Addition of the OP-1 device to the bone allograft caused an insignificant reduction in mechanical parameters. In contrast, the OP-1 device increased fixation in the ProOsteon group. The device increased ultimate shear strength of ProOsteon by 800% to a level similar to that of bone allograft (Table 1).

Histology

New bone was deposited mainly on the surface of the bone allograft (Figure 3) or ProOsteon granules (Figure 4). In the ProOsteon group, bone apposition was seen on the HA coating despite absence of bone formation in the gap close to the implant. Remnants of OP-1 collagen carrier were found in a





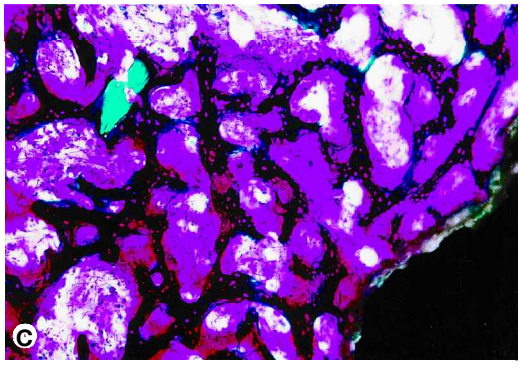
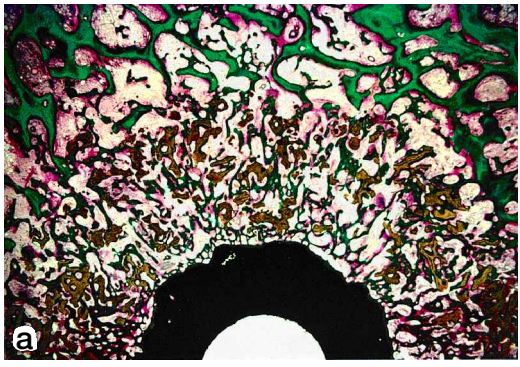


Figure 3. a. Section from bone allograft and OP-1 device group. Nearly all bone chips are resorbed, new bone forming primitive trabeculae is seen throughout the gap. ×12.5 magnification, light green, basic fucsin stain.

b. Section from the bone allograft group. Bone chips are seen in the whole gap, limited new bone formation in zone 1. ×12.5 magnification, light green, basic fucsin stain.

c. Section from bone allograft and OP-1 device group. Polarized light, a single bone chip is seen in the gap as a light green, laminar structure. Newly formed bone is darker. ×100 magnification, light green, basic fuchsin stain.



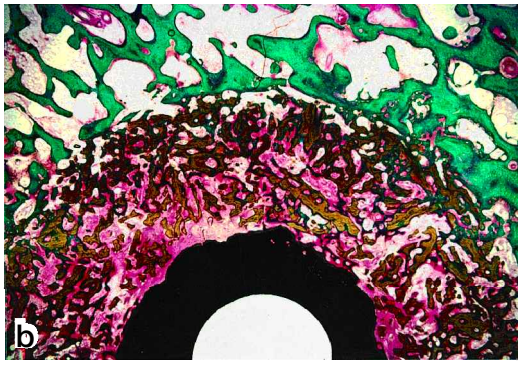


Figure 4. a. Section from ProOsteon and OP-1 device group. New bone formation in both zones, bone in contact with implant. ×12,5 magnification, light-green, basic fuchsin stain.

b. Section from the ProOsteon group. No bone formation in zone 1. ×12.5 magnification, light green, basic fuchsin stain.

few gaps. Resorption lacunae could be seen on the surface of the allograft and ProOsteon. Unmineralized tissue was mainly soft and rich in cells.

Histomorphometry

We found no significant differences in the growth of bone on the implant between the 4 groups. The OP-1 device significantly increased bone formation in the gap in both zones and in combination with both grafting materials (Table 2). In zone 1, 6 (0–13)% of woven bone was seen in ProOsteon without OP-1 as against 12 (7–20)% in bone allograft without OP-1. In zone 2, 23 (15–26)% of woven bone was deposited in ProOsteon without OP-1 compared to 16 (10–19)% in bone allograft without OP-1 ($p < 0.05$). Addition of the OP-1 device to the gap increased the volume fraction of trabecular bone at the border of the drill-hole (zone 3) from 37 (0.32–0.43) % to 44 (34–0.53)% ($p = 0.04$). Resorption of bone allograft increased significantly which resulted in more unmineralized tissue. No resorption of ProOsteon was seen (Table 3).

Discussion

HA coating, one way to enhance bony anchorage of prostheses (Kärrholm et al. 1994), can induce the healing of gaps up to 2 mm after 6 weeks (Søballe et al. 1992c) and convert fibrous tissue to bone around loaded implants (Søballe et al. 1992a, b, 1993). In this study, a control implant without graft material was not included because healing of a 3 mm gap after only 3 weeks was not expected although the implants were not coated with HA (Lind et al. 1998).

Table 2. Bone growth on implant and gap healing. Median (range)

	Allograft				ProOsteon			
	– OP-1 device		+ OP-1 device		– OP-1 device		+ OP-1 device	
	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2
Bone growth on implant	13 (0–13)		13 (3–28)		12 (0-33)		39 (0–69)	
Woven bone	12 (7–20) [bc]	16 (10–19) [bc]	20 (12–27) [ab]	32 (24–37) [ab]	6 (0–13)	23 (15-26)	25 (4–35) [a]	26 (25–32) [a]
Unmineralized tissue	54 (52–64)	60 (55–63) [bc]	69 (68–73) [abc]	64 (61–75) [abc]	56 (51–58)	37 (34–45)	42 (39–68) [a]	41 (38–45)

[a] $p < 0.05$, compared to same grafting material without OP-1
[b] $p < 0.05$, compared to ProOsteon without OP-1
[c] $p < 0.05$, compared to ProOsteon with OP-1

Table 3. Fractions of grafting material and calculation of resorption. Median (range)

	Allograft				ProOsteon			
	– OP-1 device		+ OP-1 device		– OP-1 device		+ OP-1 device	
	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2
Time zero	33 (31–39)	36 (30–40)	29 (24–37)	28 (27–34)	39 (35–43)	40 (34–44)	31 (28–34)	32 (25–34)
3 weeks	30 (27–38)	24 (20–35) [a]	9 (1–18) [a]	2 (1–7) [a]	39 (36–41)	40 (39–41)	28 (24–33)	33 (28–38)
Resorption	3 (–5–7)	12 (0–16)	20 (11–28) [b]	28 (22–29) [b]	0 (–2–3)	0 (–1–1)	4 (–2–7)	0 (–7–4)

[a] $p < 0.05$, compared to time zero
[b] $p < 0.05$, compared to same material without OP-1

Fixation of implants in the ProOsteon group without OP-1 was weaker than that of the bone allografted group with or without OP-1. This accords with Turner et al. (1993) who found limited effect of HA/TCP granules around implants that bone allograft did. When the OP-1 device was added to ProOsteon, fixation became similar to that of bone allograft. The latter device increased bone formation in the bone allograft group, but did not significantly affect fixation after 3 weeks. Our findings accord with those of previous studies where OP-1 mixed with bone allograft and inserted into created defects reduced mechanical fixation of HA-coated or uncoated implants (Lind et al. 1998, Søballe and Bechtold 1999). However, in a revision model, OP-1 increased fixation of uncoated titanium implants after 4 weeks (Søballe and Bechtold 1999). In this study with only 3 weeks of observation time, we found that the OP-1 device increases not only bone formation, but also accelerates bone graft resorption and results in significantly more unmineralized tissue. This may be why mechanical fixation in the allograft group was not increased by adding the OP-1 device.

When ProOsteon 200 granules were applied without OP-1, we found less bone formation close to the implant (zone 1) than bone allograft without OP-1. However, bone formation in the ProOsteon 200 group was significantly greater than in the allograft group in zone 2, indicating that ProOsteon 200 is a good osteoconductor (Table 2). ProOsteon is a slow resorbing bone substitute (Shimazaki and Mooney 1985, Holmes et al. 1987, el Deeb and Holmes 1989, Martin et al. 1993). Accordingly, we found no significant resorption of HA granules after 3 weeks (Table 4).

Bone formation was dramatically increased after adding the OP-1 device to ProOsteon. This correlates with a number of studies in which HA granules and other bone substitutes have been combined with various osteoinductive agents, such as BMP-3, PDGF, TGF-β or DBM at skeletal or extraskeletal sites (el Deeb et al. 1989, Ripamonti et al. 1992, Ono et al. 1996).

Histomorphometry showed a significant increase in bone formation and resorption of the bone allograft chips in the OP-1 treated group. This has previously been described, but not quantified (Salkeld et al. 1997). Remodeling of cortical grafts is initiated by resorption and followed by bone deposition. This may reduce mechanical strength. Some authors have described similar events in remodeling of cancellous bone (Einhorn 1995). Others state that cancellous graft is incorporated by a process where new bone is first deposited on the surface of the graft, which causes an initial increase in mechanical strength until the graft is remodeled (Grenga et al. 1989). Histological analyses of biopsies of morselized cancellous bone grafts impacted around endoprostheses have been studied in animals and humans (Schreurs et al. 1994, 1996, Nelissen et al. 1995). In goats, incorporation of the bone graft occurs after 6 weeks and, after 12 weeks, most of the graft is resorbed at some levels and replaced by poorly organized soft tissue infiltrated by woven bone (Schreurs et al. 1994). In humans, morselized impacted bone allograft can be found years after implantation. No evidence

has been presented that these necrotic bone chips impairs the clinical outcome. In the present study, the addition of an OP-1 device to the bone allograft increased new bone formation and graft resorption. The rate of resorption was higher than the rate of new bone formation. This mismatch causes significantly more unmineralized tissue and less mineralized tissue. Bone allograft around prostheses serves not only as a bone conductor, but also as a mechanical support for the prostheses. Uncontrolled bone graft resorption before the formation of bone may cause loss of stability of prostheses, result in micromotions and ultimately in failure.

Growth factors, such as BMP-2 and PDGF, are well known stimulators of bone formation (Arm et al. 1996, Bostrom et al. 1996, Fischgrund et al. 1997). However, BMP-2 stimulates osteoclasts in vitro (Kanatani et al. 1995) and PDGF has been associated with aseptic loosening of prostheses (Xu et al. 1998, Salcetti et al. 1997). The role of OP-1 in stimulation of osteoclasts has not been studied in vivo but an in vitro study indicates that OP-1 plays an important role in the recruitment of osteoclasts (Hentunen et al. 1995). Furthermore, preliminary results of a human trial in humans with spinal intracorporal application of the OP-1 device have shown improved bone resorption as a primary event (Laursen et al. 1999).

The OP-1 device consists of OP-1 in a bovine collagen type I carrier. Our study design does not show whether bone formation and graft resorption were stimulated by OP-1 or the collagen carrier. Bovine collagen type I stimulates human osteoblasts in vitro (Masi et al. 1992) and collagen can enhance integration of bone substitutes in vivo (Ono et al. 1995, Johnson et al. 1996). The effect, however, is limited compared to BMP and collagen (Ono et al. 1992).

We have shown that a composite of a semisynthetic bone substitute and an osteoinducting agent can be an alternative to bone allograft. However before clinical application of OP-1, in combination with bone allograft around weight-bearing endoprostheses, methods for controlling bone graft resorption should be studied.

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In memory of Dr. Lai-Martine Schiøtz.

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