Osteogenic protein-1 increases the fixation of implants grafted with morcellised bone allograft and ProOsteon bone substitute

AN EXPERIMENTAL STUDY IN DOGS

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Impacted bone allograft is often used in revision joint replacement. Hydroxyapatite granules have been suggested as a substitute or to enhance morcellised bone allograft. We hypothesised that adding osteogenic protein-1 to a composite of bone allograft and non-resorbable hydroxyapatite granules (ProOsteon) would improve the incorporation of bone and implant fixation. We also compared the response to using ProOsteon alone against bone allograft used in isolation. We implanted two non-weight-bearing hydroxyapatite-coated implants into each proximal humerus of six dogs, with each implant surrounded by a concentric 3 mm gap. These gaps were randomly allocated to four different procedures in each dog: 1) bone allograft used on its own; 2) ProOsteon used on its own; 3) allograft and ProOsteon used together; or 4) allograft and ProOsteon with the addition of osteogenic protein-1.

After three weeks osteogenic protein-1 increased bone formation and the energy absorption of implants grafted with allograft and ProOsteon. A composite of allograft, ProOsteon and osteogenic protein-1 was comparable, but not superior to, allograft used on its own.

ProOsteon alone cannot be recommended as a substitute for allograft around non-cemented implants, but should be used to extend the volume of the graft, preferably with the addition of a growth factor.

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Restoration of bone stock is often a major challenge in the revision of a loose total hip replacement (THR). Morcellised fresh-frozen femoral head allograft has given good results when impacted to restore bone in femoral and acetabular bone defects.[1,2] Because of the risk of disease transmission and the limited availability of bone allograft, alternatives are being investigated. Calcium phosphate-based bone substitutes such as hydroxyapatite (HA) have been used to restore bone defects in the acetabulum and the proximal femur.[3] Mechanically, a composite of calcium phosphate granules and morcellised allograft might provide adequate initial stability to be used to graft the femur.[4] However, little is known about the biological consequences of adding such bone substitute to bone allograft. One problem associated with the use of ceramics in this setting is the lack of osteoinduction. Adding bone growth factors such as osteogenic protein-1 (OP-1) (bone morphogenic protein-7) to a composite of morcellised bone allograft and HA granules might increase the biological activity and accelerate bone repair and fixation of the implant.

The aim of this study was to investigate whether HA granules could be used as a substitute for bone allograft or be added to it to expand the available volume. We also investigated the effect of OP-1 on a composite of bone allograft/HA granules. Evaluation was based on mechanical testing of fixation of the implant and studying bone histomorphometry in a canine model.

Materials and Methods

The study was conducted using eight skeletally-mature Labrador dogs. One additional dog served as the source of the allograft. The dogs were bred for research and had a mean age of 14 months (12 to 14) and a mean weight of 29.3 kg (27.5 to 30.3). The protocol was accepted by the Danish Animal Research Committee and the experiment was conducted in accordance with Danish law.

The proximal humeri of each dog were used for the implantation of two small metal implants, leaving a 3 mm gap between the implant and the host bone (Fig. 1).

The four gaps in each dog were randomised to be grafted in group 1 with bone allograft; in

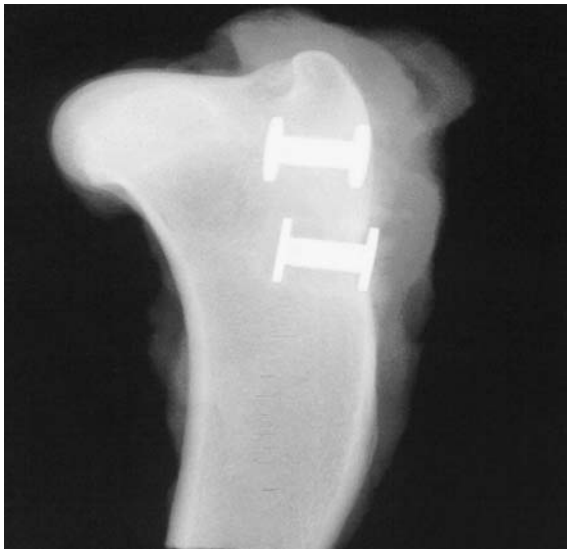

Fig. 1a

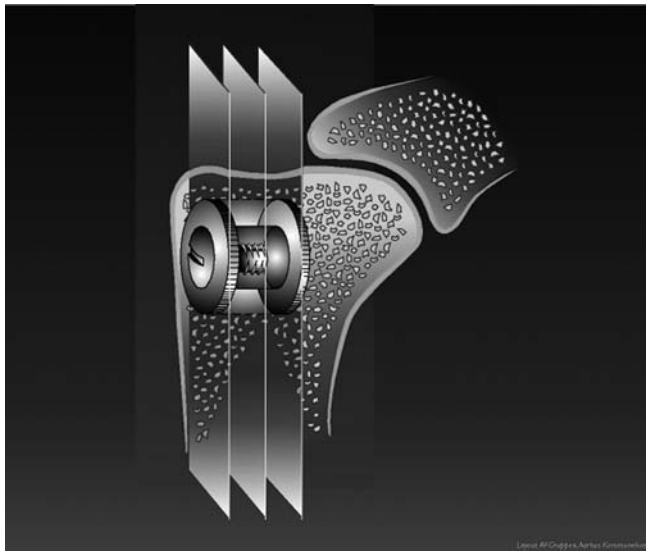

Fig. 1b

a) Radiograph and b) diagram of the proximal humerus showing the placement of the implants. Each implant was inserted into the proximal humerus. After three weeks, the implants were divided into two; one half was used for histomorphometry, the other half was used for mechanical testing.

group 2 with ProOsteon; in group 3 with allograft mixed with ProOsteon and in group 4 with allograft mixed with ProOsteon to which OP-1 had been added. Allograft and ProOsteon were mixed in equal volumes in groups 3 and 4. All procedures were carried out by the same operator (TBJ). The intention was to blind all evaluations but as the grafting materials were easily distinguishable, the operation and histomorphometry could not be performed blinded. The grafting materials were weighted pre-operatively and packed into containers. The weight necessary to fill the gaps was determined in pilot studies, and the materials were mixed to be as homogenous as possible.

Grafting materials. *Bone allograft.* The proximal humerus and the proximal and distal femur were harvested from a dog not included in the study. The bone was frozen at -80°C for two weeks, then thawed and the soft tissue and cartilage removed prior to milling to produce chips that ranged between less than 1 mm and 7 mm. The graft was weighed into portions, packed into sterile containers and re-frozen at -80°C. All bone preparation was undertaken under strict aseptic conditions in an operating theatre, and bacterial cultures were taken to exclude any contamination of the allograft.

ProOsteon 200 (Interpore, Irvine, California) is a coralline porous HA bone substitute approved by the Food and Drug Administration (FDA). It was provided as granules with a diameter of 425 μm to 1000 μm and a mean porous diameter of 200 μm.[5] Before the operation, it was weighed, placed in containers and autoclaved according to the manufacturer's instructions.

OP-1 was delivered as a preparation consisting of 2.5 mg recombinant human OP-1 mixed with 1 g of bovine type I collagen (Stryker Biotech, West Lebanon, New Hampshire). A dose of 300 μg OP-1 with 120 mg of collagen carrier was mixed with ProOsteon/bone allograft prior to grafting, reducing the volume of ProOsteon/allograft by 10% to accommodate the volume of OP-1.

Implants. Cylindrical titanium alloy (Ti-6A1-4V) implants with a plasma-sprayed titanium porous coating were manufactured by Biomet (Warsaw, Indiana). A 50 μm thick HA coating with a calcium to phosphate ratio of 1.67 was plasma-sprayed on the porous titanium alloy coating by BioInterfaces Inc (San Diego, California). The final size of the implants was 10 mm long and 5 mm in diameter. The measured surface roughness (Ra) of the HA-coated implants was 41 μm using a previously described method.[6] The implants were sterilised by gamma irradiation. A standardised 3 mm gap was maintained around the implant on insertion into the prepared bed by a footplate and a washer (Fig. 2).

Surgery. Under general anaesthesia and observing aseptic precautions the proximal humeri of each dog were exposed by a lateral extra-articular approach. A 1.8 mm guide wire was inserted into the bone and two 11 mm diameter holes were hand-drilled using a cannulated drill. The most proximal implant was inserted immediately distal to the greater tuberosity and the additional implant was placed 9 mm more distally (Fig. 1). Before and after each operation, 1 g ampicillin (Anhypen, Gist-Brocades, Delft, The Netherlands) was administered intravenously. In order to harvest the experimental material the dogs were sedated with

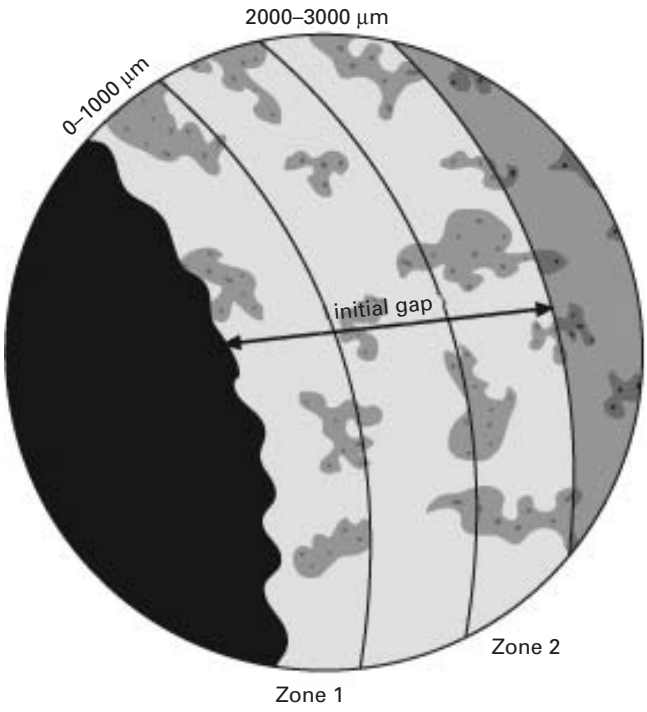

Fig. 2

Volume fractions of bone, bone allograft and other tissue were determined in two zones. Zone 1: 0 mm to 1 mm from implant surface (implant is black), zone 2: 2 mm to 3 mm from the implanted surface (the surrounding bone is dark grey).

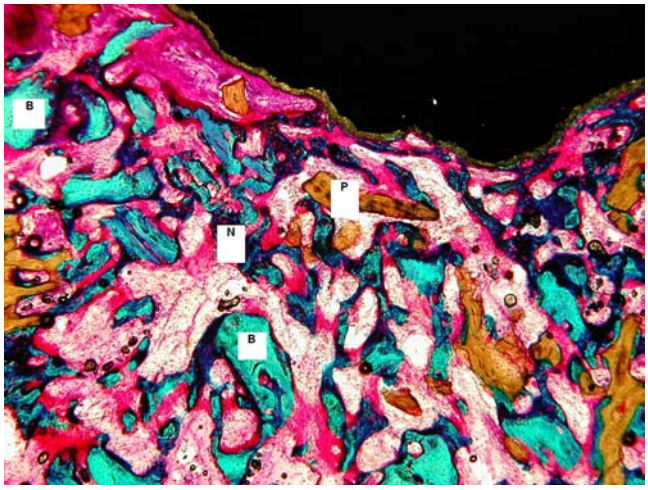

Fig. 3a

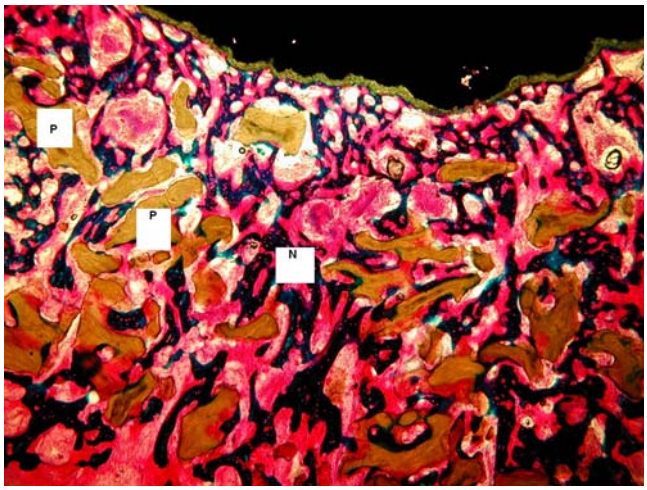

Fig. 3b

Photomicrographs of a) bone allograft/ProOsteon group without OP-1 device. Bone formation (N) is seen close to the implant. Bone allograft chips (B) as well as ProOsteon (P) are seen in the gap. b) Bone allograft/ProOsteon group with OP-1 device. Much woven bone (N) is seen and ProOsteon is not resorbed. No bone allograft chips can be detected (undecalcified sections; staining: basic fuchsin and light green; calcified tissue is green/blue, soft tissue is red, ProOsteon is brown. Magnification x 50).

methohexital (Brietal, Lilly, Denmark) and killed with an overdose of potassium chloride three weeks after the implantation.

Preparation of tissue samples. The retained proximal parts of the humeri were stored at -20°C. Sections perpendicular to the long axis of the implant were made using a water-cooled diamond bandsaw (Exact Apparatbau, Norderstedt, Germany) as previously described.[7] As a first step, the most superficial 1 mm of the specimen was removed and discarded. The following section, with a thickness of 5 mm, was divided in half along the axis of the implant. One half was randomly selected for histomorphometry. The other was stored for an experiment on immunohistology which is not included in the current report. The remainder of the specimen, closest to the footplate, was stored at -20°C and used for mechanical testing.

Histomorphometry. The specimens were dehydrated in 70% to 100% ethanol containing 0.4% basic fuchsin and subsequently embedded in methylmethacrylate (Techovit 7200 VLC; Exact Apparatbau). Four horizontal sections 50 μm in thickness were cut with a microtome (MeProTech, Heerhugowaard, The Netherlands) and surface counterstained with 2% light green for two minutes,[8] to colour all mineralised tissue green. Bone allograft was distinguished from newly-formed bone by the lamellar light-green structure with empty lacunae, compared to the darker woven structure of newly-formed bone. ProOsteon stained brown and the collagen carrier from the OP-1 device had a reddish colour with this technique (Fig. 3).

The field of view obtained with the microscope was transmitted to a computer screen. Histomorphometry was performed using a software program (CAST-Grid, Olympus, Denmark) which allowed the application of grid-lines and points.[9] Bone ingrowth, the proportion of woven bone, grafting material and non-mineralised tissue present in the gap around the implant were determined on each section. Bone ingrowth was evaluated by the linear intercept technique.[9] Approximately 250 interceptions on the surface of each implant were counted and the ingrowth calculated as bone cover in relation to total surface area and expressed as

a percentage. The gap surrounding the implant was divided into two zones, 0 mm to 1 mm (zone 1), and 2 mm to 3 mm (zone 2) from the implant surface, with 275 points examined in each zone at a magnification of 100× (Fig. 1). All specimens were evaluated blindly.

Reproducibility. The histomorphometric measurements were performed twice on all sections from the ProOsteon group and the allograft groups (a total of 12 implants) at an interval of approximately two years by the same person (TBJ). Reproducibility (intra-observer variation) was calculated as the coefficient of variation.[10]

Mechanical testing. Push-out testing was performed using an Instron universal testing machine (Instron Ltd., High Wycombe, United Kingdom). Specimens were centralised over a metal platform with a circular hole supporting the bone specimen to within 500 μm of the interface.[11] The implant was displaced at a velocity of 5 mm/minute and load-deformation curves were obtained on an *x-y* recorder (PM 8043, Phillips, Eindhoven, The Netherlands). Ultimate shear strength, apparent stiffness and energy absorption were estimated from the load-displacement curves, as previously described.[9,11]

Statistical analysis. All data are presented as mean values with standard deviation (SD) in brackets. A two-way analysis of variance was applied and the Student-Newman-Keuls test was used for all pairwise comparisons between the different groups. The proportions of woven bone, grafting material and non-mineralised tissue in the gaps were not compared statistically. A p-value < 0.05 was considered significant.

Results

All bacterial cultures from morcellised bone allograft were sterile. Two dogs were not fully mobile after two weeks and they were therefore killed before the observation period of three weeks and excluded from the study. Investigation showed no sign of infection around the implants.

Histology. *Histomorphological findings.* Most bone formation and bone graft resorption was seen at the margins of the gaps, except in the OP-1-treated specimens, where bone formation and graft resorption had also occurred close to the implant surface (Fig. 3). Remnants of the OP-1 collagen carrier were found in a few gaps. Lacunae due to resorption of material were recognised on the surface of allograft and ProOsteon. Non-mineralised tissue was mainly soft and cell-rich, with no signs of infection.

Histomorphometric findings. Implants not treated with OP-1 had a mean bone ingrowth of 33% (SD 28), whereas those with OP-1 had a mean ingrowth of 48% (SD 28). These differences were not statistically significant (p = 0.50). Where ProOsteon was used alone and when combined with bone allograft there was significantly less new bone formation in zone 1 than in the other treatment groups. Only minor differences in bone formation were seen in zone 2 (Table I). The mean proportion of bone allograft after three weeks in the graft and ProOsteon group was dramatically reduced, from 9% (SD 1) to 2% (SD 1) in zone 1 and from 12% (SD 4) to 0% (SD 0) in zone 2 when OP-1 was added (Table II) but with little difference in the mean proportion of ProOsteon that could be detected (Table II).

Mechanical tests. Implants grafted with bone allograft and ProOsteon with OP-1 had significantly higher energy absorption than implants grafted with bone allografts and ProOsteon or ProOsteon alone (Table III). The same tendency was seen in the other mechanical parameters. Analysis of variance found no significant difference for stiffness among the treatment groups (p = 0.08).

Reproducibility. The coefficient of variation was highest in the bone allografted group when the proportion of graft (allograft or ProOsteon) and woven bone was estimated.

Discussion

Aseptic loosening of failed total joint replacements is associated with resorption of the surrounding bone. Impaction of bone allograft has been used to restore the bone bed for more than a decade, with variable results.[2,12] Allograft bone is associated with the risk of transmitting diseases. By observing appropriate screening protocols, the risk of viral transmission is small, with only one reported case of HIV infection attributed to donated bone allograft.[13]

The use of bone substitutes as an alternative to bone graft or as a bone-graft enhancer has previously been studied clinically and experimentally *in vivo* by Jensen et al[14] and Turner et al,[15] and *in vitro* by Blom et al[4] and Verdonschot et al.[16] Japanese workers have published good clinical results using HA granules as a substitute to bone graft in the femur and acetabulum in the revision of failed hip replacements.[3,17] Retrieval studies showed that the majority of the HA granules were incorporated into remodelled trabeculae at a maximum follow-up of ten years.[18] These promising results are in contrast to the present, and other experimental studies.[14,15] This may be a function of difference in duration of implantation before analysis was undertaken, with Oonishi et al[18] making observations at up to ten years and our own being made after only three weeks. We chose a short observation time for several reasons. The incorporation of impacted bone allograft is fastest during the first six months in humans,[19] and radiological examinations show little change after two years.[20] Furthermore, the early micromovement of implants is a predictor of early failure.[21] Because bone remodelling in dogs is faster than in humans, we believe that an observation period of three weeks is clinically relevant.

In vitro studies have shown excellent stability of femoral stems grafted with a composite of hydroxyapatite and tricalcium phosphate granules and bone graft.[4,16] We showed that the addition of ProOsteon bone substitute to bone allograft significantly reduced new bone formation, but that the addition of OP-1 overcame this problem and significantly increased bone formation in zone 1 after three weeks. This suggests that a composite of HA granules and‡

Table I. Bone ingrowth and proportions of woven formed bone and soft tissue in the gap in percentages (mean (SD))

		Woven bone		Soft tissue	
	Bone ingrowth	Zone 1	Zone 2	Zone 1	Zone 2
Allograft (n = 6)	*32* (28)	*27* (6)*,†	*34* (4)	*51* (8)	*44* (7)*,†
ProOsteon (n = 6)	*36* (36)	*18* (7)‡	*31* (5)	*52* (11)	*36* (6)
Allograft + ProOsteon (n = 6)	*31* (24)	*21* (7)‡	*31* (4)	*51* (6)	*37* (4)
Allograft + ProOsteon + OP-1¶(n = 6)	*48* (22)	*31* (4)	*33* (3)	*55* (5)	*48* (4)

Statistical analysis applying the Student-Newman-Keuls test
*, $p < 0.05$ compared with ProOsteon
†, $p < 0.05$ compared with allograft + ProOsteon
‡, $p < 0.05$ compared with allograft + ProOsteon + OP-1
¶, OP-1, osteogenic protein 1

Table II. Proportions of grafting materials at three weeks, in percentages (mean (SD)). No statistical comparison was performed

	Allograft		ProOsteon		Allograft + ProOsteon		Allograft + ProOsteon + OP-1*	
	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2
Allograft (n = 6)	*22* (5)	*23* (6)	-	-	9 (1)	*12* (4)	*2* (1)	*0* (0)
ProOsteon (n = 6)	-	-	*31* (10)	*33* (4)	*19* (3)	*20* (3)	*14* (13)	*20* (3)

*OP-1, osteogenic protein 1

Table III. Mechanical fixation of the implants based on a push-out test (mean (SD))

Group	Energy absorption (J/m^2)	Ultimate shear strength (MPa)	Apparent stiffness (MPa/mm)
Allograft (n = 6)	299 (287)	2.35 (1.87)	22.5 (23.5)
ProOsteon (n = 6)	79 (115)*	0.72 (0.94)*	5.4 (6.7)
Allograft + ProOsteon (n = 6)	165 (137)*	1.80 (1.49)	15.5 (12.5)
Allograft + ProOsteon + OP-1† (n = 6)	543 (211)	3.66 (1.58)	26.2 (17.9)
ANOVA‡	p = 0.03	p = 0.04	p = 0.08

*, $p < 0.05$ compared with allograft + ProOsteon OP-1
†, OP-1, osteogenic protein 1
‡, ANOVA, analysis of variance

Table IV. Coefficient of variation based on double measurements at a two-year interval (*percentage*)

		Woven bone		Soft tissue		Graft/ProOsteon	
	Bone ingrowth	Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2
Allograft (n = 6)	*6*	*9*	*5*	*3*	*4*	*7*	*12*
ProOsteon (n = 6)	*9*	*6*	*4*	*2*	*5*	*5*	*6*

bone allograft should be modified biologically to reach the same level of bone incorporation as bone allograft used alone.

In the present study, the fraction of bone allograft was much smaller in the OP-1-treated group than in the non-OP-1-group (Table II), indicating that OP-1 increases not only bone formation but also resorption of bone allograft. One major risk of using OP-1 in combination with bone allograft around weight-bearing implants is uncontrolled graft resorption.[14,22-25] The possible stimulatory effect of OP-1 on bone resorption is supported clinically by the experience of Laursen et al,[26] who found it to be a primary event when OP-1 was used to treat unstable thoracolumbar burst fractures in humans. This might explain early failure in revision of THR where bone allograft and OP-1 had been used in combination.[27] Adding non-resorbable HA granules to bone allograft might be a possible way of ensuring the stability of the implant during remodelling of the bone graft, but our model using a non-weight-bearing implant does not allow us to make any further comment.

In the present study, we quantified the proportions of woven newly-formed bone, soft tissue, bone graft and ProOsteon. We significantly increased implant fixation and bone formation by adding OP-1 to a composite of bone allograft and non-resorbable ProOsteon, but none of the groups was better than bone allograft used on its own.

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In memory of Dr Lai-Martine Sciøtz (1972 to 2000).

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References

1. **Schreurs BW, Slooff TJ, Buma P, Gardeniers JW, Huiskes R.** Acetabular reconstruction with impacted morsellised cancellous bone graft and cement: a 10- to 15-year follow-up of 60 revision arthroplasties. *J Bone Joint Surg [Br]* 1998;80-B:391-5.
2. **Lind M, Krarup N, Mikkelsen S, Horlyck E.** Exchange impaction allografting for femoral revision hip arthroplasty: results in 87 cases after 3.6 years' follow-up. *J Arthroplasty* 2002;17:158-64.
3. **Oonishi H, Iwaki Y, Kin N, et al.** Hydroxyapatite in revision of total hip replacements with massive acetabular defects: 4- to 10-year clinical results. *J Bone Joint Surg [Br]* 1997;79-B:87-92.
4. **Blom AW, Grimm B, Miles AW, Cunningham JL, Learmonth ID.** Subsidence in impaction grafting: the effect of adding ceramic bone graft extender to bone. *Proc Inst Mech Eng [H]* 2002;216:265-70.
5. **Holmes RE, Bucholz RW, Mooney V.** Porous hydroxyapatite as a bone graft substitute in diaphyseal defects: a histometric study. *J Orthop Res* 1987;5:114-21.
6. **Soballe K, Hansen ES, B-Rasmussen H, Jorgensen PH, Bunger C.** Tissue ingrowth into titanium and hydroxyapatite-coated implants during stable and unstable mechanical conditions. *J Orthop Res* 1992;10:285-99.
7. **Jensen TB, Rahbek O, Overgaard S, Soballe K.** No effect of platelet-rich plasma with frozen or processed bone allograft around noncemented implants. *Int Orthop* 2005;29:67-72.
8. **Gotfredsen K, Budtz-Jørgensen E, Jensen LL.** Preparation and staining of sections containing titanium-implants. *Stain Tech* 1989;64:121-7.
9. **Overgaard S, Lind M, Glerup H, et al.** Hydroxyapatite and fluorapitite coatings for fixation of weight loaded implants. *Clin Orthop* 1997;386:286-96.
10. **Therkelsen AJ.** Vurdering af metodenøjagtighed på basis af en serie dobbeltbestemmelser. In: Therkelsen AJ, ed. *Medicinsk statistik.* Aarhus, FADL,s forlag, 1983:79-81.
11. **Soballe K, Hansen ES, Brockstedt-Rasmussen H, Pedersen CM, Bunger C.** Bone graft incorporation around titanium-alloy- and hydroxyapatite-coated implants in dogs. *Clin Orthop* 1992;274:282-93.
12. **Eldridge JD, Smith EJ, Hubble MJ, Whitehouse SL, Learmonth ID.** Massive early subsidence following femoral impaction grafting. *J Arthroplasty* 1997;12:535-40.
13. **Asselmeier MA, Caspari RB, Bottenfield S.** A review of allograft processing and sterilization techniques and their role in transmission of the human immunodeficiency virus. *Am J Sports Med* 1993;21:170-5.
14. **Jensen TB, Overgaard S, Lind M, et al.** Osteogenic protein 1 device increases bone formation and bone graft resorption around cementless implants. *Acta Orthop Scand* 2002;73:31-9.
15. **Turner TM, Urban RM, Sumner DR, Galante JO.** Revision, without cement, of aseptically loose, cemented total hip prostheses: quantitative comparison of the effects of four types of medullary treatment on bone ingrowth in a canine model. *J Bone Joint Surg [Am]* 1993;75-A:845-62.
16. **Verdonschot N, van Hal CT, Schreurs BW, et al.** Time-dependent mechanical properties of HA/TCP particles in relation to morsellized bone grafts for use in impaction grafting. *J Biomed Mater Res* 2001;58:599-604.
17. **Oonishi H, Kadoya Y, Iwaki H, Kin N.** Total hip arthroplasty with a modified cementing technique using hydroxyapatite granules. *J Arthroplasty* 2001;16:784-9.
18. **Oonishi H, Kadoya Y, Iwaki H, Kin N.** Hydroxyapatite granules interposed at bone-cement interface in total hip replacements: histological study of retrieved specimens. *J Biomed Mater Res* 2000;53:174-80.
19. **Linder L.** Cancellous impaction grafting in the human femur: histological and radiographic observations in 6 autopsy femurs and 8 biopsies. *Acta Orthop Scand* 2000;71:543-52.
20. **Elting JJ, Mikhail WE, Zicat BA, et al.** Preliminary report of impaction grafting for exchange femoral arthroplasty. *Clin Orthop* 1995;319:159-67.
21. **Karrholm J, Borssen B, Lowenhielm G, Snorrason F.** Does early micromotion of femoral stem prostheses matter?: 4-7-year stereoradiographic follow-up of 84 cemented prostheses. *J Bone Joint Surg [Br]* 1994;76-B:912-17.
22. **McGee MA, Findlay DM, Howie DW, et al.** The use of OP-1 in femoral impaction grafting in a sheep model. *J Orthop Res* 2004;22:1008-15.
23. **Salkeld SL, 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-A:803-16.
24. **Soballe K, Jensen TB, Mouzin O, Kidder L, Bechtold JE.** Differential effect of a bone morphogenetic protein-7 (OP-1) on primary and revision loaded, stable implants with allograft. *J Biomed Mater Res A* 2004;71:569-76.
25. **Tagil M, Jeppsson C, Wang JS, Aspenberg P.** No augmentation of morselized and impacted bone graft by OP-1 in a weight-bearing model. *Acta Orthop Scand* 2003;74:742-8.
26. **Laursen M, Hoy K, Hansen ES, et al.** Recombinant bone morphogenetic protein-7 as an intracorporal bone growth stimulator in unstable thoracolumbar burst fractures in humans: preliminary results. *Eur Spine J* 1999;8:485-90.
27. **Karrholm J, Hourigan P, Timperley J, Razaznejad R.** Mixing bone graft with OP-1 does not improve cup or stem fixation in revision surgery of the hip: 5-year follow-up of 10 acetabular and 11 femoral study cases and 40 control cases. *Acta Orthop* 2006;77:39-48.