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

AN EXPERIMENTAL STUDY IN DOGS


From Department of Orthopaedics,
Århus University Hospital, Denmark

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.

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. 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. Mechanically, a composite of calcium phosphate granules and morcellised allograft might provide adequate initial stability to be used to graft the femur. 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 osteoinductivity. 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...
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 coraline 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-6Al-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 (Anhpyen, Gist-Brocades, Delft, The Netherlands) was administered intravenously. In order to harvest the experimental material the dogs were sedated with...
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 Apparaturbau, 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 Apparaturbau). 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. 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 al14 and Turner et al,15 and in vitro by Blom et al14 and Verdon schot 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.5,17 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 al18 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...
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.

The authors thank Biomet Inc. for providing the implants, Interpore Inc. for providing ProOsteon 200 granules, and Stryker Biotech for providing the OP-1.

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

<table>
<thead>
<tr>
<th></th>
<th>Bone ingrowth</th>
<th>Woven bone</th>
<th>Soft tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone 1</td>
<td>Zone 2</td>
<td>Zone 1</td>
</tr>
<tr>
<td>Allograft (n = 6)</td>
<td>32 (28)</td>
<td>27 (6)(^1)</td>
<td>34 (4)</td>
</tr>
<tr>
<td>ProOsteon (n = 6)</td>
<td>36 (36)</td>
<td>18 (7)(^1)</td>
<td>31 (5)</td>
</tr>
<tr>
<td>Allograft + ProOsteon (n = 6)</td>
<td>31 (24)</td>
<td>21 (7)(^1)</td>
<td>31 (4)</td>
</tr>
<tr>
<td>Allograft + ProOsteon + OP-1(^\dag) (n = 6)</td>
<td>48 (22)</td>
<td>31 (4)</td>
<td>33 (3)</td>
</tr>
</tbody>
</table>

Statistical analysis applying the Student-Newman-Keuls test
\(^*, p < 0.05\) compared with ProOsteon
\(^†, p < 0.05\) compared with allograft + ProOsteon
\(^\dag, p < 0.05\) compared with allograft + ProOsteon + OP-1

\(\dag\), OP-1, osteogenic protein 1

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

<table>
<thead>
<tr>
<th></th>
<th>Allograft</th>
<th>ProOsteon</th>
<th>Allograft + ProOsteon</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Zone 1</td>
<td>Zone 2</td>
<td>Zone 1</td>
</tr>
<tr>
<td>Allograft (n = 6)</td>
<td>22 (5)</td>
<td>23 (6)</td>
<td>-</td>
</tr>
<tr>
<td>ProOsteon (n = 6)</td>
<td>-</td>
<td>31 (10)</td>
<td>33 (4)</td>
</tr>
<tr>
<td>Allograft + ProOsteon (n = 6)</td>
<td>9 (1)</td>
<td>12 (4)</td>
<td>2 (1)</td>
</tr>
</tbody>
</table>

\(*\), OP-1, osteogenic protein 1

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

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

\(^*, p < 0.05\) compared with allograft + ProOsteon OP-1
\(^\dag, p < 0.05\) compared with allograft + ProOsteon + OP-1

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Woven bone</th>
<th>Soft tissue</th>
<th>Graft/ProOsteon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone 1</td>
<td>Zone 2</td>
<td>Zone 1</td>
</tr>
<tr>
<td>Allograft (n = 6)</td>
<td>6</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>ProOsteon (n = 6)</td>
<td>9</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

\^, ANOVA, analysis of variance
Jane Pauli and Annette Milton kindly assisted at the operations and prepared the sections. Thanks to Flemming Melsen, MD, PhD, Institute of Pathology, for histological advice. The Danish Rheumatism Association, the Danish Medical Research Council, Institute of Experimental Clinical Research, the Velux Foundation, Beckett Foundation and the University of Århus supported the study financially.

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

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References


