Paper III

OP-1 increases strength of morselized bone allograft after 3 weeks

Experimental study in canines

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The mechanical properties of bone allograft and HA granules with and without Osteogenic Protein-1 was investigated 3 weeks after impaction around noncemented implants.
A hole of 11 mm in diameter was drilled in the medial and lateral condyle in both knees of 6 labrador dogs. One HA-coated titanium implant 6 mm in diameter was centralized in each hole leaving a gap of 3 mm. The four gaps in each dog were grafted with bone allograft or HA granules with or without OP-1. 300 μg OP-1 was delivered in a bovine collagen type I carrier (OP-1 device).
A special set-up was developed to determine the mechanical properties of the graft after 3 weeks. The impacted gaps were centralized over a fixture with a hole of 11.3 mm in diameter. A piston 10.0 mm in diameter loaded the impacted graft. By adding OP-1 to bone allograft, energy absorption was increased from 25 N to 53 N ($p<0.05$). In the ProOsten grafted group, energy absorption was increased from 6 N to 33 N ($p<0.05$) by adding OP-1.
In conclusion, OP-1 device improved the mechanical properties of impacted bone allograft and HA granules. HA alone showed inferior properties.

Introduction

Morselized, impacted bone is widely used in the revision of failed total joint replacements. Examination of retrieved impacted bone allograft from humans show, that osseointegration of impacted bone allograft is unpredictable and bone chips embedded in fibrous tissue can be found after years . Therefore the addition of growth factors capable of stimulating bone formation has been suggested [3,5,8,9]. OP-1 (BMP-7) is a member of the bone morphogenic protein family and a strong stimulator of bone healing [6]. OP-1 has recently been postulated to cause failure of bone grafted femoral stems in canines [5] and humans [1]. One possible explanation is, that OP-1 accelerates resorption of bone graft and thus loss of mechanical fixation[6].
Limited access, risks of transmission of diseases and immunological rejection of bone allograft advocates for the use of bone graft substitutes such as HA-granules. However such granules are are only osteoconductive and less bioactive compared to bone allograft [6]. Adding OP-1 device to HA-granules dramatically increases bone ingrowth [6].
The objects of the present study was to compare the mechanical properties of impacted bone allograft chips and HA granules and to describe the mechanical consequences of adding OP-1 to those two grafting materials *in vivo.*

MATERIALS AND METHODS

Design:

Data in this article are obtained from specimens used in a previous study describing implant fixation and bone formation around noncemented implants [6]. Six Labrador dogs with an age of 14 months and an average weight of 27.5 kg (25-30kg) were used. One additional dog served as donor of bone allograft. The protocol was accepted by the Danish Committee for Animal Research Committee and animal handling was performed according to Danish laws for research handling. Observation time was three weeks. Implants were inserted in overdrilled holes extraarticularly in each medial and lateral femoral condyle creating concentric 3 mm gaps. The four gaps in each dog surrounding the implants were block randomised to one of the following treatment groups: Group 1: Allograft, group 2: ProOsteon, group 3: Allograft+OP-1 device, group 4: ProOsteon+OP-1 device.

Grafting materials:

Bone allograft: The proximal humerus, proximal and distal femur were harvested after sacrificing the donor dog. The bone was stored at -80°C and later thawed and processed. Soft tissue and

cartilage was removed prior to morsellizing. The bone was morsellized into bone chips using a standard bone mill (Biomet® Inc, Warsaw, IN, US) [6].

HA-granules: ProOsteon 200 granules with an average porous diameter of 200 µm (Interpore, Irvine, US) were used. ProOsteon 200 was delivered as granules with a diameter of 425-1000µm. Before operation, it was weighed into portions and autoclaved [6].

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

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 with a length of 10 mm and a final diameter of 5 mm. Implants were porous coated and HA plasma sprayed. A standardised 3 mm gap was obtained by a footplate and a washer.

Surgery

Anaesthesia was induced by intravenous Brietal (10mg/kg) and maintained by halothane. Unloaded implants were inserted extraarticularly into the medial and lateral condyles in both knees as previously described [15]. 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 according to the treatment groups described. Before and after each operation, 1 gram Ampicillin (Anhypen; Gist-Brocades, Delft, Holland) was administered. The dogs were killed after Methohexital (Brietal;Lilly;Denmark) sedation with an overdose of KCl.

Preparation of tissue samples

The distal femurs were harvested and stored at -20°C. Sections perpendicular to the long axis of each implant were made on a water cooled diamond band saw (Exact, Apparatebau, Norderstedt, Germany). The most superficial 5.0 mm were prepared for histomorphometry for another study [6], the lower 3.5 mm were stored at -20°C and used for mechanical testing.

Mechanical testing

Mechanical tests were done using an Instron Universal test machine (Instron Ltd. High Wycombe, UK). Following mechanical test of the bone/implant interface [6] (Figure 1a), the specimen were centralized over a hole of 11.3 mm in diameter. A piston 10.0 mm in diameter loaded the bone in the gap at a displacement rate of 5 mm/minute (Figure 1b). A preload of 2 N defined the contact position for the start of the test. The implant was displaced at a velocity of 5 mm/min and load-deformation curves were obtained. Ultimate shear strength (σ_u) was determined from the maximal force (F) and was calculated as $\sigma_u=F/L$ where L is 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 $E = (\delta F/L)/\delta L$. Energy absorption was calculated from the area beneath the curve until failure.

Statistical analysis

Data are presented as median and range in brackets (min-max). After application of Kruskal-Wallis One Way Analysis of Variance on Ranks (ANOVA on ranks), groups were pairwise compared

using Student-Newmann-Keuls test. P-values less than 0.05 (two-tailed) were considered significant.

Results

A preload of 2 N could be obtained from all samples and there were no drop-outs. Two of the specimen grafted with ProOsteon without OP-1 did not describe a typical failure curve. Failure was seen in the interface between the incorporated graft and the border of the drill hole.
Adding OP-1 device to bone allograft significantly increased energy absorption appr 100% (table I). ProOsteon alone had inferior mechanical properties with an energy absorption only 25% of that in the allografted group. ProOsteon+OP-1 had an umtimate shear strength comparable with allograft+OP-1 but energy absorption was appr. 40% lower. The differences in stiffness between the four groups were not statistical significant (ANOVA on ranks=0.55)

Discussion

Impacted morselized bone allograft chips are commonly used in revision of failed total hip replacements. Success of such revisions are dependent on stability of the prosthesis at the time of operation and during bone incorporation and remodelling of the graft. Since early subsidence is an important predictor of longevity, early failure during remodelling of the bone graft could lead to failure. Biopsies of morselized cancellous bone grafts impacted around endoprostheses have been studied in animals and humans [10,13,14]. In humans, necrotic morselized bone allograft incorporated by fibrous tissue are found long time after implantation.
Addition of bone growth factors capable of stimulating bone growth is one approach to enhance bone incorporation of impacted bone allograft. Previously, growth factors such as OP-1 (BMP-7), BMP-2, TGF-β2 and bFGF has been mixed with bone allograft. Overall OP-1 and BMP-2 increase bone formation [6,7], number of osteoclasts [7] and graft resorption [6].
We previously applied a uni-directional load on the implant parallel to the long axis of the implant to study the mechanical properties of implant-bone interface. This test is a destruction test with failure usually at the bonding between bone and HA coating [11]. The gap is supported to within a distance of 500 μm from the implant and no load is applied on the graft close to the border of the drill hole, tested in the present study.
Previously we found, that addition of OP-1 to impacted bone allograft increased new bone formation by almost 100% ($P<0.05$)[6]. However volume fraction of bone allograft decreased from 25% to 3% leading to an overall increase in non-mineralised tissue ($p<0.05$)[6].
The mechanical test of the bone grafted gaps in the present study showed that ultimate shear strength and energy absorption was dramatically increased. This indicates, that it is not the total fraction of mineralised tissue but the fraction of new bone that predicts the stability of the construct. Tagil et al showed, that impacted graft penetrated by fibrous tissue had double compression strength compared to freshly impacted graft [16] and that impacted graft might not necessarily be invaded by bone to ensure mechanical stability. This study indicates, that bone ingrowth is favourable.
One question still to be answered is, if OP-1 increases bone graft resorption prior to bone formation. In that case, we might find a situation with little bone graft remained but still no new bone formation to ensure the mechanical stability. A study with more time points could answer that question.
ProOsteon is very brittle (strength and stiffness is higher but energy absorption is low compared to cancellous bone). The mechanical behaviour of impacted ProOsteon granules have previously been investigated showing, that bone incorporated HA granules had higher stiffness and shear strength compared to impacted bone graft or bone [17]. Addition of OP-1 device to ProOsteon had huge effect on all mechanical parameters. This was expected since OP-1 significantly increased bone formation

and did not increase resorption of ProOsteon which is considered to be a non-resorable bone substitute.
ProOsteon without OP-1 showed inferior mechanical properties compared to the three other groups. This was expected since histology showed inferior bone ingrowth and implant fixation[6].
ProOsteon with OP-1 had ultimate shear strength comparable to allograft with or without OP-1. However since stiffness was 30% higher (NS), energy absorption was significantly lower than bone allograft alone. Since ProOsteon is considered non-resorbable, a composite of ProOsteon and bone will probably never get the same biomechanical characteristics as native bone.
In conclusion, this study shows, that addition of OP-1 to morselized bone allograft and ProOsteon increases the mechanical properties after 3 weeks. This was expected in the ProOsteon group, but not predictable in the bone grafted group since OP-1 decreased the total amount of mineralised tissue. Since impaction grafting in general shows good clinical results even though incorporation is not predictable, the use of OP-1 should be used with care.

Figure legends and table

Figure 1. Two mechanical tests were performed on the bone-implant specimen. A) Implant fixation was tested by centralizing the implant with a diameter of 6 mm over a hole with a diameter of 7 mm and descriped in another study [6]. B) The grafted 11 mm gap was centralized over a hole of 11.3 mm. A piston with a diameter of 10.0 mm applied load on the gap.

Table I: Mechanical data (median (range),n=6)

Group	**Ultimate shear strength (MPa)**	**Energy abs. (J/m^2)**	**Apparent Stiffness (MPa/ mm)**
Allograft	60 (6-102)	25 (1-38)a	190 (29-328)
ProOsteon	35 (10-57)a	6 (2-20)a	165 (11-323)
Allograft+OP-1	84 (57-109)	53 (43-81)a	198 (49-292)
ProOsteon+OP-1	87 (53-106)	33 (24-76)a	283 (61-372)

a: $p<0.05$ compared to three other groups

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