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Biomechanical and histological evaluation of a hydroxyapatite-coated titanium femoral stem fixed with an intramedullary morsellized bone grafting technique: an animal experiment on goats

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To reconstruct femoral intramedullary bone-stock loss in revision surgery of failed total hip arthroplasties, morsellized trabecular bone grafts can be used. In 14 goats a noncemented hydroxyapatite-coated titanium stem was fixed within a circumferential construction of bone allografts. After 6 or 12 wk, four goats were used for mechanical tests and three for histology. The stability of the stems relative to the bone was determined in a loading experiment with Roentgenstereo-Photogrammetric Analysis (RSA). Owing to two loosening and two fractures, only one 6-wk specimen and three 12-wk specimens were available for mechanical testing. The prostheses were very stable at 12 wk. The most important movements were axial rotation (maximal 0.17° at 800 N) and subsidence (maximal 0.036 mm at 800 N). After unloading, there was 40–60% elastic recovery. Histological examination showed revascularization and remodelling of the graft in all the specimens investigated. At the graft site, bone apposition and bone resorption had resulted in a mixture of graft and new bone. Bone incorporation was mainly seen in the proximal areas. Graft lysis was evident in the midshaft region and at distal levels around the prostheses. Copyright © 1996 Elsevier Science Limited

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Despite the impressive results of cemented total hip arthroplasty, about 10% of operations have to be revised within 10 yr¹. Aseptic loosening, which is the major long-term cause for revision, is associated with migration of the implant, the formation of a radiolucent line on X-rays, and bone stock loss. Although cementless prostheses have yielded good short-term results^{2,3}, they can also produce lysis and loosening⁴.

The main problem encountered during femoral revision is a loss of intramedullary bone stock, which is caused by the loosening process itself and by the removal of the prosthesis and cement. The results of femoral revision after simply filling the defect with bone cement are unsatisfactory, even with modern cementing techniques or in combination with long stems^{5–8}. Several grafting techniques to reconstruct the femur, using different types of bone graft, have been described^{9–14}. Based on the poor results of revision

with structural grafts at the acetabular site, we advise against using this type of graft¹⁵. Some authors have advocated the use of only noncemented techniques in cemented revision cases¹⁶, but the results of these noncemented revisions are unsatisfactory, with femoral loosening up to 9.5% after 1 yr^{17–19}.

Since 1979, in our department, severe cases of acetabular bone stock loss have been successfully restored with a bone grafting technique using impacted morsellized trabecular bone chips²⁰. In 1988, a special set of instruments was developed for the femoral application of this procedure. Intramedullary reconstruction of the endosteal wall could be achieved with morsellized bone chips.

In the present study, the viability of this technique in combination with an experimental noncemented hydroxyapatite (HA) coated stem was investigated in an animal experiment. The biomechanical stability of the stem 6 and 12 wk postoperatively was determined with roentgenstereo-photogrammetric analysis (RSA)²¹. The histological analysis focussed on the rate of

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consolidation, the rate of incorporation of the graft and the interface area between the prosthesis and the bone.

MATERIALS AND METHODS

Fourteen adult goats (*Capra Hircus Sana*) underwent surgery on the right hip using general anaesthesia and standard disinfection techniques. The dorsolateral approach was used and the hip was luxated. After resection of the femoral head, the femoral canal was prepared using hand reamers (9–14 mm). The canal was cleaned and an appropriately sized bone cement plug (AlloPro size 3.5–5) was screwed on to a metal rod (diameter 10 mm) which was then introduced into the medullary canal (see *Figure 1* for a diagram of the technique). The space between this rod and the cortical bone (2–4 mm) was filled with chip-like trabecular grafts in a retrograde fashion. Trabecular bone grafts were harvested from donor goats under sterile conditions. Donor sites were the sternum, the distal femur, proximal tibia and humeral head. Perioperative bacterial cultures were taken. All the swabs of the implanted allografts were negative. Grafts were stored at -80° until implantation. The maximal storage time was 6 months. Before use, the grafts were thawed at room temperature. Using a special set of instruments, consisting of several sizes of tubes sliding over a central metal rod, the grafts could be compressed axially and radially (*Figure 1*). After the

filling process had been completed, the central metal rod was removed, leaving a central cavity surrounded by impacted graft. A noncemented titanium prosthesis was inserted into this cavity (Ti 6Al-4V ELI Canine Hip Prosthesis by Osteonics, fully coated with HA powder, CaP ratio 1.66, plasmasprayed; thickness 40–60 μ m; crystallinity *ca.* 70%; *Figure 2*. The HA coating was applied by CAM b.v.; de Groot *et al.*²²). An acrylic strut containing a tantalum pellet was glued to the tip of the prosthesis prior to insertion for the RSA measurements. The diameter of the modular femoral head was 22 or 26 mm (CoCr bearing in T 799 alloy). After the operation, the goats were kept in a hammock for 1–2 d, then they were transferred to cages which allowed free walking. X-rays were taken immediately after the operation, if appropriate at 6 wk postoperatively, and after the goats were killed. Loading patterns of the goats were graded weekly using the scoring system of function²³, explained in the Results section. Goats were killed after 6 (7 goats) or 12 (7 goats) wk by an overdose of sodium pentobarbital. In each group four goats were used for biomechanical RSA studies and three for histological investigation. The motion of the stems relative to the cortical bone was measured with the RSA method described by Selvik²¹. The femora for the biomechanical studies were freshly harvested and stored at -80°C ready for testing. After thawing, the femora were resected just above the condyles and partly embedded in polymethylmethacry-

Figure 1 Schematic drawing of the graft impaction technique using a special set of instruments. A bone cement plug (a) is screwed on to a metal rod (b) and introduced into the canal. The space between the metal rod and the cortical bone is filled with trabecular bone grafts (c). These grafts are impacted using metal tubes which slide over the central rod. Different types of tube are used for axial (d) and radial (e) impaction of the grafts.

Figure 2 The titanium prosthesis fully coated with HA.

late (PMMA). Tantalum pellets contained in acrylic struts were attached proximally and distally to the medial and lateral sides of the cortical bone, three at each location. Two small acrylic struts containing three pellets each were glued to the medial and lateral aspects of the head of the prosthesis. In this way, two sets of three pellets proximally and one single pellet distally defined the position of the prosthesis. The implanted prostheses were then loaded in an MTS testing machine. Relative to the vertical position, the femora were tilted 15° in the lateral direction and endorotated 45° , in order to obtain a physiological load on the femoral head^{23,24}. The load was applied stepwise from 0 to 200, 500, and 800 (± 10) N (Figure 3). After each loading step, the load was kept constant for 10 min. Before loading, after each loading step and again 10 min after the final unloading, stereoroentgenograms were taken. These were measured using an Aristomat digitizer. The 3D pellet positions at all the time periods during the loading cycle were determined using the RSA computer system. To increase accuracy, all the roentgenograms were measured five times and the results were averaged. This method produced translations of the prosthesis relative to the cortical bone along the X-axis (lateral-medial translation), Y-axis (axial translation, i.e. subsidence), and Z-axis (antero-posterior translation). Rotations around the X-axis (rotation in the sagittal plane), Y-axis (horizontal plane) and Z-axis (frontal plane) were calculated. The coordinate system is depicted in Figure 4.

To allow qualitative assessment of bone remodelling, all the goats selected for the histological study received intravital fluorochromes. We used terramycin (d 8–12, $25 \text{ mg kg}^{-1} \text{ d}^{-1}$), alizaron complexon (6-wk group, d 23–27; 12-wk group, d 49–53, $30 \text{ mg kg}^{-1} \text{ d}^{-1}$) and calcein green (6-wk group, d 38–42; 12-wk group d 80–84, $20 \text{ mg kg}^{-1} \text{ d}^{-1}$)^{25–27}. In order to visualize the revascularization of the graft, the legs were perfused with Micropaque[®] according to the microangiographic procedure of Rhinelander and Baragry²⁸. Both femora were harvested after careful exarticulation and fixed in a mixture of ethanol and formalin. After contact-roentgenograms had been taken, the femora were cut into slices of

Figure 4 Femur with bone grafts and a prosthesis. The laboratory coordinate system is shown.

3 mm with the prosthesis still *in situ*. The sectioning scheme allowed observations along the entire length of the prosthesis. To study the bone-prosthesis interface and the integrity of the HA layer, the slices were studied using routine, fluorescence and confocal microscopy. To facilitate further standard histological analysis, the titanium prosthesis core was removed. For microangiography, slices were decalcified in formic acid under radiological control. For fluorescence microscopy, slices were embedded in PMMA and sectioned ($30 \mu\text{m}$) on a rotating water-cooled diamond saw (Leitz 1600)^{29,30}. For routine histology, slices were decalcified in EDTA, embedded in PMMA, section ($7 \mu\text{m}$) and stained with HE.

RESULTS

Clinical observations

The average weight of the goats was 62.2 kg (48–77 kg). There were no perioperative losses. The mean operation time was 3.5 h (3 h 15 min–4 h 15 min). Two goats in the 6-wk group were lost to follow-up owing to a fracture of the operated femur at the tip of the prosthesis. In one case a spontaneous fracture occurred 5 wk after the operation (G6-C), in the other case the fracture occurred after trauma during transportation from one cage to another (G6-D). The loading patterns

Figure 3 The loading regime. Roentgenstereograms were taken 10 min after the increase in load. The arrows indicate when roentgenstereograms were taken.

of the goats are graphically represented in Figure 5A and B. All the goats loaded the prosthesis during walking, except for G6-B. At the time of being killed, three prostheses showed rotational instability (G6-B, G6-E, G12-D).

Radiological observations during the clinical phase

Most of the prostheses were placed in a neutral position, although some were slightly in varus (see Figure 8C). In one goat (G12-E) a fracture occurred in the calcar zone which was evident during the operation. However, fracture healing was seen. Subsidence was estimated on the AP and lateral standard radiograms. Owing to standardization problems of the clinical radiograms, precise measurements were hampered. All but four cases showed gross subsidence of several mm of the prosthesis at 6 wk relative to the immediate postoperative position. No subsidence was observed in any of

the goats in the period 6–12 wk. On the postoperative radiograms the area in which the graft was located was seen as a homogeneous radio-opaque structure. In most of the cases after 6 wk and in all of the cases after 12 wk, this area was more radiolucent (see Figure 8B, C).

RSA measurements

The standard deviations for the displacements in the RSA study were estimated to be 0.036 mm and 0.07° for translations and rotations, respectively. Owing to the two fractures and one evident loosening, only one specimen was left for biomechanical testing at 6 wk (Figures 6A, 7B). In the 12-wk group, three specimens could be used; one was lost owing to evident loosening (Figures 6B, 7B). Any translations and rotations were small. Figures 6 and 7 show a graphical representation of the Y-translation, resulting in subsidence of the prosthesis relative to the cortical bone, and the psi rotation around the Y-axis, resulting in axial rotation of the prosthesis. Rotations and translations in the other directions were generally much smaller (except

Loading pattern
Follow-up 6 weeks

a

Loading pattern
Follow-up 12 weeks

b

Figure 5 Grading of postoperative weight-bearing according to Ypma²³. Goats were scored weekly; the scores at 2, 4, 6, and 12 wk are presented. 0, Not used at all; 1, supported incidentally; 2, loaded in standing position and incidentally while walking; 3, loaded in standing position and walking, but with a limp; 4, normal standing and walking.

Rotations noncemented prosthesis After 6 weeks implantation

a

Rotations noncemented prosthesis After 12 weeks implantation

b

Figure 6 Axial rotations found for the specimens in: a, the 6-wk group; and b, the 12-wk group, from unloaded to stepwise increases in load, back to unloaded.

Subsidence noncemented prosthesis After 6 weeks implantation

a

Subsidence noncemented prosthesis After 12 weeks implantation

b

Figure 7 Subsidence found in: **a**, the 6-wk specimens; and **b**, the 12-wk group, from unloaded to stepwise increases in load, back to unloaded.

for G12-B; translation $\times$ direction under 800 N was 0.115 mm). In all the cases subsidence increased with load. After unloading, elastic recovery occurred, which resulted in very small permanent translations and rotations. After 5 additional loading cycles, the 12-wk specimens showed an average subsidence of 0.009 mm and an average additional rotation of 0.03°.

Histological analysis

The loosening of specimen G6-E was due to a histologically proven infection. It was discarded from further histological analysis.

The roentgenphotos of the whole bones suggested local differences in the incorporation of the graft into a new trabecular structure (Figure 8B, C). Radiolucent areas were found, particularly at midshaft levels, which indicated that complete incorporation had not taken place (Figure 8B). Therefore, proximal, midshaft and distal levels were studied separately. Changes in the architecture of the graft were confirmed in detail on the contact-roentgenograms of the slices (Figure 8D,

E) and histologically. In three cases microfractures were found in the proximal cortical bone.

At locations where no vascular invasion had taken place, the original medullary fat had been replaced by a loosely organized fibrin clot, and the graft consisted of large pieces of trabecular bone which showed microfractures due to the impaction process (Figure 8G, H). Histologically, the grafted bone could be easily recognized by the empty osteocyte lacunae or, if present, the pycnotic appearance of the osteocytes (Figure 9A, B, D). The graft was found to be infiltrated by vascular elements, loose connective tissue, macrophages, osteoblastic and osteoclastic bone cells (Figure 8J). This revascularization and ossification front could be followed by the polychrome sequential labelling. The first activity of this front was seen in the endosteal cortex, in time penetrating to the more central parts of the grafts. Distally, we observed that revascularization of the graft took a few weeks longer, owing to damage to the compact cortical bone induced by the operation. Many osteoclasts and osteoblasts were involved in the process of bone lysis, formation and incorporation of the graft (Figure 9B, D, H). The bony structure formed was a mixture of necrotic bone graft and woven trabecular bone, which had been laid down on the graft (Figure 9B, D).

At proximal levels, revascularization, incorporation and remodelling of the grafts were seen at the lateral trochanteric site after 6 wk, and the trabeculae had formed interconnections with the pre-existing host bone at the corners of the prosthesis (Figure 9A, B). At 12 wk, revascularization and incorporation were also seen on the medial side, although these were less pronounced than on the lateral side. Both fluorescence microscopy and confocal microscopy showed very close contact between the graft and the HA (Figure 9C). The distance of new bone to the metal ranged between 40 and 60 μ m, which indicated that there was direct bone-HA contact. This was confirmed by the histological sections after the prosthesis had been removed. A layer of HA was present at the locations where bone ingrowth had occurred into the HA (Figure 9E). Although there was fracture healing in G12-E, there was no proximal bone-HA contact. Instead a 300- μ m thick fibrous layer had formed (Figure 9F), with loose HA crystals at the interface. Trabecular bone had developed in a shell around this fibrous interface. Polarized light showed that the orientation of the collagen fibres was perpendicular to the surface of the prosthesis.

After 12 wk most of the graft had disappeared at midshaft levels (Figures 8E, 9G, H). The process of osteolysis took slightly longer than 6 wk to start. Many osteoclastic cells were present and were responsible for resorbing the graft (Figure 9H), which had been replaced by loosely organized fibrous tissue. From the cortical wall this fibrous tissue was found to be infiltrated by woven callous bone, which had never made direct contact with the prosthesis (Figure 9G).

After 12 wk some areas of the graft were still present in the original non-revascularized form at distal levels around the prosthesis (Figure 8G, H). Similar phenomena were also observed at midshaft levels. After revascularization, graft lysis predominated

Figure 8 A–C, Roentgenograms of A, control status, and implant status after B, and C, 12-wk. Note the proximal trabecular bone in A and the different zones of bone incorporation in B. The roentgenological appearance of the proximal graft has changed from a diffuse appearance to a trabecular structure (large arrow). At midshaft levels (small arrows) the graft is radiolucent. D–F, Roentgenograms of thick sections at D, proximal, E, mid-shaft, and F, distal levels along the prosthesis in the 12-wk group. G, Graft after impaction but before incorporation. $\times 15$. H, Enlargement of the encircled area in G. $\times 45$. J, Granulation reaction associated with wound healing and graft incorporation after 6 wk. Note the many monocytic cells. $\times 250$.

Figure 9 A, HE-stained section of the proximal lateral bony structure after 12 wk. Note the connections between the host cortical bone and the prosthesis (removed for histotechnical reasons). $\times 15$. B, Enlargement of the encircled area in A. Note the bone graft (G) with empty osteocyte lacunae and the new bone with viable osteocytes (NB). $\times 100$. C, Fluorescence microscopy of the proximal bone-prosthesis interface taken with a confocal microscope. The HA layer itself is hardly visible. $\times 50$. D, HE-stained section showing consolidation of the graft to the necrotic cortical host bone (NCB). Note the remnants of graft (G) and new bone (NB). E, Sawed section showing bonding of the bone to the layer HA. $\times 140$. F, Section through the proximal part of cortical bone, showing interface formation. $\times 15$. G, HE-stained section of the midshaft after 6 wk with newly formed woven bone (W), loose connective tissue (LCT) and the graft (G). H, Enlargement of the encircled area in G showing osteoclastic graft lysis. $\times 180$. J, Contact site of the tip of the prosthesis with the cortical bone. New bone (NB) had formed between the prosthesis and the host cortical bone (CB). $\times 25$. K, Same location but with fluorescence microscopy. Note the intense labelling of the new bone with calcein green between the cortical bone (CB) and the HA coating (HA) of the prosthesis (P). $\times 30$.

(Figure 8F). Microscopy showed that a bridge of new bone had formed between the tip of the prosthesis and the cortical bone, especially if the prosthesis had been placed with some degree of valgus (Figure 9J, K).

DISCUSSION

The animal model selected for this experiment is thought to be quite pertinent to the human situation, although it is not a true revision model. The femoral canal of the goat is wide enough to perform the grafting technique; the hard and smooth endosteal surface has very little trabecular bone and is similar to the sclerotic endost usually encountered in revision surgery of failed hip prostheses. The goat is fairly adaptable and shows normal loading patterns soon after hip surgery, in contrast to dogs. The stem shape is similar to that of human prostheses. Because of the superior bone inductive capacities³¹⁻³⁴, a HA coating was applied which had the same properties as the one used for human prosthesis. HA leads to the enhanced fixation of load-bearing implants³⁵. Recent studies of loaded and initially unstable implants have confirmed the effect of HA coatings, even in the presence of a motion-induced fibrous membrane around the implant³⁶. HA has a superior gap healing influence to a distance of 2 mm, but the influence of HA on the incorporation of unloaded trabecular allografts is not clear³⁷. No comparable data are available in the literature on a loaded model.

The loads applied in the biomechanical testing procedure were realistic at 129% of body weight, and were even high relative to the loads of 110% of body weight measured *in vivo* in sheep²⁴. Based on the same measurements²⁴, the load direction produced axial, torsional and bending components which are all essential to assess the stability of the stem³⁸⁻⁴⁰. The RSA technique provided accurate 3D motions of the stem relative to the bone and proved to be easy to use.

Although immunotyping of the goats was considered, it was not applied owing to difficulties expected in the interpretation of results with such a relatively small series of animals^{41,42}. To prevent bias due to immunoresponse, however, the donor goats were obtained from other breeders than the receptor goats, so close consanguineous relationships were excluded⁴³.

Our failure rate was relatively high. Only four of the eight specimens could be used for biomechanical testing and another specimen intended for histological analysis showed loosening due to infection. During impaction of the grafts a fracture appeared peroperatively in one femur, because the femur of the goat is very hard and brittle. However, after initial subsidence the prosthesis gained secondary stability. No more fractures were observed in the clinical roentgenstudy, although the microradiograms taken in the histological study frequently showed repaired microfractures of cortical bone. It is possible that these intraoperatively induced microfractures were responsible for one fracture and the two loosening. The other fracture was caused by trauma.

Subsidence upon maximal loading was very

consistent in the 12-wk specimens at 15–35 μm , of which 40–60% was permanent after the first loading cycle. The 6-wk results indicated a trend towards better stability with increasing time. After five additional loading cycles, the 12-wk specimens showed additional permanent subsidence of 9 μm . Although these values were small relative to the precision of the RSA method, they were very consistent and demonstrated that the prostheses were stable after 12 wk when heavily loaded. The rotations showed less consistent values, but the trends pointed in the same direction. These elastic and permanent relative displacements were very small compared with the direct postoperative situation in which elastic subsidence of up to 2955 μm and axial rotations of up to 6.8° were measured upon maximal loading; in one case 2722 μm of subsidence did not recover⁴⁴. In a comparable previous study the stability of cemented stems and morsellized allografts was estimated⁴⁵. After 12 wk the cemented stems showed subsidence up to 160 μm , of which 78 μm was permanent after the first loading cycle. Thus, these noncemented stems proved to be very stable if a secondary stability could be achieved. We did not find any data in the literature on the stability of noncemented stems fixed within bone grafts. However, to facilitate bone ingrowth in porous coated prostheses, the maximal relative motion allowed between the prosthesis and the bone is 28–40 μm ^{38,46}. The estimated micromotions were within the scope of those suggested in the literature to permit bone ingrowth, which was confirmed by histology. We found that the graft had become revascularized and incorporated into a new bony structure, which could be followed by fluochrome labelling. The most incorporation was seen in the proximal lateral area where there was sufficient direct bone–HA contact to transfer stress from the prosthesis to the bone. Graft lysis was seen at midshaft and distal levels. Although the repair of the endosteal microcirculation was not complete on the endosteal surfaces at the midshaft and distal levels, even at 12 wk, it cannot explain this lysis. Regarding the histological ingrowth pattern, this prosthesis will probably generate bone and interface stresses like those estimated for a partly proximally coated stem in a FEM⁴⁷. The stress pattern (with reduction especially at a distal level) could be the explanation for the level-dependent differences in graft incorporation, suggesting that graft incorporation is partly dependent on load. Proximal bridges of trabecular bone to the corners of the prosthesis were also found in a retrieval study⁴⁸. There was good contact between the incorporated graft and the HA coating, especially in the proximal region. However, in one case there was fibrous tissue contact between the prosthesis and the bone. Based on retrieval experiments, it was stated that limited bone ingrowth with extensive fibrous tissue seems to be an effective means of stabilizing primary porous-coated femoral stems⁴⁹.

In contrast to structural bone grafts¹⁵, the use of this revision technique of impacted morsellized trabecular intramedullary allografts in combination with cement is becoming quite popular, both acetabularly and femoral^{20,50}. Morsellized trabecular bone grafts are

frequently used to fill gaps around noncemented stems. The use of intramedullary femoral bone grafts with cementless devices has been described, although never in an impacted form and loaded by a stem^{14,51,52}.

CONCLUSIONS

To cope with the severe femoral bone stock loss encountered in revision surgery, we used impacted trabecular bone grafts in combination with a hydroxyapatite-coated titanium stem. In this first experimental animal study, the results indicate that this technique has a high complication rate. However, it has been shown that impacted grafts can sustain the loaded stems and that incorporation of the graft occurs with a biomechanically stable implant. The technique allows gradual graft incorporation and stability, but more investigations are needed before its introduction into clinical practice.

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