Porous titanium particles for acetabular reconstruction in total hip replacement show extensive bony armoring after 15 weeks

A loaded in vivo study in 10 goats

Lucas H B Walschot[1], René Aquarius[2], Nico Verdonschot[2,3], Pieter Buma[2], and B Willem Schreurs[1]

[1]Department of Orthopaedics, Radboud University Medical Centre, Nijmegen; [2]Department of Orthopaedics, Orthopaedic Research Laboratory, Radboud University Medical Centre, Nijmegen; [3]Laboratory of Biomechanical Engineering, University of Twente, Enschede, the Netherlands.
Correspondence: wim.schreurs@radboudumc.nl

Background and purpose — The bone impaction grafting technique restores bone defects in total hip replacement. Porous titanium particles (TiPs) are deformable, like bone particles, and offer better primary stability. We addressed the following questions in this animal study: are impacted TiPs osteoconductive under loaded conditions; do released micro-particles accelerate wear; and are systemic titanium blood levels elevated after implantation of TiPs?

Animals and methods — An AAOS type-III defect was created in the right acetabulum of 10 goats weighing 63 (SD 6) kg, and reconstructed with calcium phosphate-coated TiPs and a cemented polyethylene cup. A stem with a cobalt chrome head was cemented in the femur. The goats were killed after 15 weeks. Blood samples were taken pre- and postoperatively.

Results — The TiP-graft layer measured 5.6 (SD 0.8) mm with a mean bone ingrowth distance of 2.8 (SD 0.8) mm. Cement penetrated 0.9 (0.3–1.9) mm into the TiPs. 1 reconstruction showed minimal cement penetration (0.3 mm) and failed at the cement-TiP interface. There were no signs of accelerated wear, metallic particle debris, or osteolysis. Median systemic titanium concentrations increased on a log-linear scale from 0.5 (0.3–1.1) parts per billion (ppb) to 0.9 (0.5–2.8) ppb (p = 0.01).

Interpretation — Adequate cement pressurization is advocated for impaction grafting with TiPs. After implantation, calcium phosphate-coated TiPs were osteoconductive under loaded conditions and caused an increase in systemic titanium concentrations. However, absolute levels remained low. There were no signs of accelerated wear. A clinical pilot study should be performed to prove that application in humans is safe in the long term.

■

A major challenge in both primary and revision total hip replacement (THR) is dealing with acetabular bone deficiencies. A total hip replacement has a limited lifespan and it usually fails at the acetabular side (Swedish Hip Arthroplasty Register, Annual Report 2008). Different surgical techniques can be used to deal with acetabular defects. Only bone impaction grafting (BIG) combines all the desired features of anatomic positioning of the center of rotation, bone stock regeneration, and good long-term survival (Gie et al. 1993, Paprosky et al. 2005, Flecher et al. 2008, Bicanic et al. 2009). However, BIG becomes more challenging in large defects and high failure rates have been reported in AAOS type-III and -IV defects (Garcia and Marti-Gonzalez 2004, van Haaren et al. 2007, Lee and Nam 2011). More extensive impaction grafting reconstructions have thicker graft layers and biomechanical studies have shown that failure usually occurs within the graft layer (Arts et al. 2005a, 2005b). In clinical studies of BIG, radiographic follow-up at 5 years showed only loosening in cups with high rates of initial migration and rotation as measured by radiostereometric analysis (Karrholm et al. 2006, Ornstein et al. 2006). Thus, the key to success with the impaction grafting technique is to maintain sufficient primary stability throughout the revitalization phase, which is characterized by revascularization with partial graft resorption and subsequent temporarily mechanical weakening (van der Donk et al. 2002, Karrholm et al. 2006).

Calcium phosphate particles increase the primary stability of the graft layer (Bolder et al. 2002). Recently, good medium-term clinical results have been published for acetabular defect reconstruction with a mixture of ceramic and bone

particles and also with ceramic particles alone in combination with a non-cemented cup (Whitehouse et al. 2013). However, the inferior handling characteristics and the associated risk of fracture of the surrounding bone (Arts et al. 2005, van Haaren et al. 2005) started the search for a more ductile material. Titanium particles have been applied as a gap filler with a pneumatic oscillation technique to insert an uncemented femoral stem (Alffram et al. 2007, Turner et al. 2007). The titanium particles used in these studies were small (1.5–2.0 mm diameter) and relatively dense. In order to apply titanium particles in acetabular and femoral impaction grafting, we selected highly porous titanium particles (TiPs) with a larger diameter (2.0–5.0 mm) for preclinical testing. These TiPs are deformable, like cancellous bone particles, and result in reconstructions with superior mechanical stability (Aquarius et al. 2009, Walschot et al. 2010, Walschot et al. 2013). Furthermore, impacted TiPs with a coating of calcium phosphate are osteoconductive, like impacted cancellous bone particles, under non-loaded conditions (Walschot et al. 2012). We evaluated the use of calcium phosphate-coated TiPs in a loaded goat defect model. More specifically, we tested the following hypotheses: (1) An impacted layer of coated and impacted TiP offers adequate primary stability for osteoconduction under loaded conditions. (2) In the short term, the amount of titanium micro-particles released by TiPs will not cause adverse tissue reactions or increased wear. (3) Implantation of a large surface of titanium can increase postoperative systemic titanium concentrations relative to preoperative levels.

Materials, animals, and methods

Materials

The porous titanium particles (TiPs) used in this study (Hereford Metal Powder Company Ltd., Hereford, UK) are produced during the purification of titanium (Ti) through titanium tetrachloride ($TiCl_4$). This process creates highly porous grade-1, commercially pure titanium (99.67% Ti). The bulk material is mechanically crumbled into smaller particles, resulting in porous titanium particles (TiPs) with a wide range of sizes. One particular size range was used: particles could pass through a sieve with 4.0-mm pores but were stopped by a sieve with 2.8-mm pores. Sieved TiPs were cleaned to remove possible particulate and chemical pollution (procedure number PS03-016; CAM Implants B.V., Leiden, the Netherlands). The density of a volume of non-impacted TiPs measured 0.50 g/mL. Individual particles have an interconnected surface porosity of 83% (2%) (Walschot et al. 2012). The calcium phosphate coating is made of carbonated apatite and is relatively thin (10–40 μm) (Figure 1). The coating was applied by submersion of TiPs in saturated calcium phosphate-containing solutions at 37°C and 50°C with subsequent drying at 50°C according to a previously published protocol (Leeuwenburgh et al. 2001, Walschot et al. 2012). Coated TiPs were sterilized
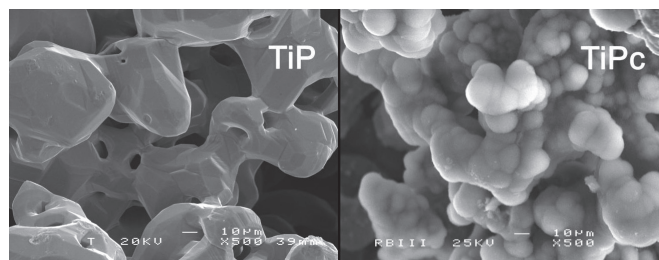

Figure 1. Left panel: Porous titanium particle (TiP) before coating. Right panel: TiP coated with carbonated apatite (SEM, 500×).

by 50 kGray gamma radiation prior to implantation (Isotron Nederland B.V., Ede, the Netherlands).

Animals and surgical technique

We used 10 mature Dutch milk goats weighing 63 (SD 6) kg (*Capra hircus sanus;* Central Animal Laboratory of the University of Nijmegen, the Netherlands). Preoperative management consisted of antibiotic prophylaxis (enrofloxacin, 0.2 mL/kg intramuscularly) and administration of a non-steroidal anti-inflammatory drug (flunixin, 1 mg/kg intramuscularly) and an opioid (buprenorphine, 5 μg/kg intramuscularly) for pain. The goats were anesthetized with pentobarbital (30 mg/kg intravenously) and isoflurane (2.5% isoflurane in an oxygen/nitro-oxygen mixture, by inhalation). Surgery was performed on the right acetabulum with the animals lying on their left side. The incision site was shaved and cleaned with povidone-iodine. Thereafter, a C-shaped incision was used to approach the right hip from the anterolateral side. The anterolateral gluteal muscles and endorotators were loosened from the femur and retracted, the joint capsule was opened with a T-shaped incision, and the femoral head was dislocated. Next, a femoral neck osteotomy was performed. The acetabulum was reamed to a diameter of 32 mm. Then the superolateral and anteromedial rims were removed with a reamer and a power drill to simulate a large combined cavitary and segmental defect (AAOS type III). The acetabulum was reconstructed with a stainless-steel mesh (X-Change; Stryker Orthopaedics, Newbury, UK), which was secured to the outer side of the pelvic bone with 4–5 AO bone screws (3.5 mm in diameter; 10–20 mm in length; Synthes, Switzerland) (Figure 2A). Small burr holes (2 mm) were made in the dense bone areas of the medial acetabular wall to facilitate vascularization of the bone graft. After a trial testing of the cup, the defect was reconstructed with TiPs with X-change comparable revision instruments (hemi-elliptical and hemispherical impactors varying in diameter from 14 mm to 32 mm) (Figure 2B). This resulted in a reconstructed hemispherical defect with a diameter of 32 mm (Figure 2C). Next, bone cement (Simplex; Stryker Orthopaedics, Limerick, Ireland) was introduced into the defect 4 min after mixing the powder with the monomer, and then pressurized for 2 min. An Exeter sheep polyethylene cup (inner diameter, 22.2 mm; outer diameter, 29 mm; Stryker
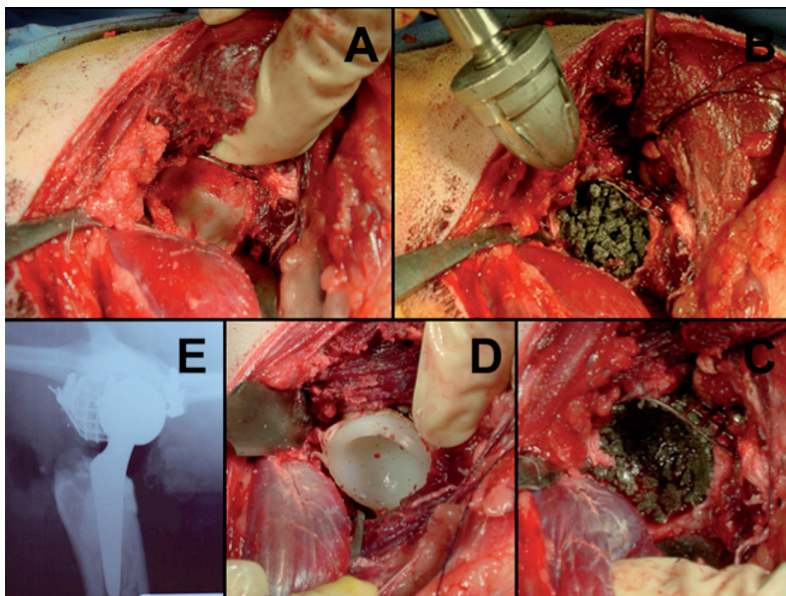
Figure 2. A. to E. Reconstruction of an acetabular AAOS type-III defect (panel A) with TiPs (B), impaction grafting (C), and a cemented downsized Exeter hip prosthesis (D and E).
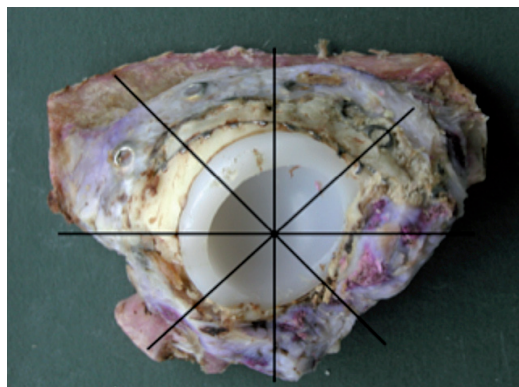

Figure 3. Retrieved acetabular reconstruction fifteen weeks after implantation. The black lines indicate the orientation and location of the sections used for histomorphometry. Superolaterally, the rim of the metal mesh is visible, as are the heads of the screws used for initial fixation of the mesh.

Orthopaedics, Newbury, UK) was inserted 6 min after mixing the cement (Figure 2D). The anti-dislocation rim of the cup often had to be downsized manually to accommodate the defect. After the acetabular cement had set, the femoral shaft was opened and prepared with broaches. The femoral canal was lavaged and bone cement was injected retrogradely 3.5 min after mixing. A polished V40 Exeter sheep stem (made of cobalt chrome (CoCr) with a corresponding V40 22.2 mm CoCr femoral head; Stryker Orthopaedics, Newbury, UK) was inserted 5 min after mixing the bone cement. After the cement had set, the hip was reduced. Next, the abductors and endorotators were re-attached and the soft tissues were closed in layers. A radiograph was taken of the hip (Figure 2E).

Postoperatively, the goats were placed in a supporting hammock for 2 weeks, which allowed full weight bearing. They received 3 doses of ampicillin after the implantation (15 mg/kg intramuscularly) and also flunixin (75 mg/24 h, intramuscularly for 3 days) and buprenorphine (0.3 mg/12 h intramuscularly for 2 days) for pain suppression. After 2 weeks, the goats were housed in an outdoor pen with ample space to walk around. Postoperatively, fluorochromes were administered at 4 weeks (tetracycline, 15 mg/kg/24h intramuscularly for 3 days), 8 weeks (calcein green, 20 mg/kg/24h intramuscularly for 3 days), and 15 weeks (alizarin, 20 mg/kg/24 h intramuscularly for 3 days) to monitor bone ingrowth distance over time. All procedures were approved by the Animal Ethics Committee of the University of Nijmegen (DEC number 2006-025, project number 21044, December 18, 2006).

Histomorphometry

The goats were killed with an overdose of pentobarbital (60 mg/kg intravenously). Standard radiographs were taken from the implant sites to verify the implant position and to exclude fractures and dislocations. After fifteen 15 weeks, both the femur and the reconstructed acetabulum were harvested, cleaned of soft tissue, and fixed for at least 10 days in a 4% buffered formaldehyde solution at 4°C. A diamond saw was used to section the harvested reconstructions into radially orientated slices (Figure 3). The first sectioning split the reconstruction, enabling removal of the 2 halves of the polyethylene cup without damaging the articular surface or the cement-graft layer interface. Then the 2 halves of the reconstruction were sectioned further. The slices were left non-decalcified and were embedded in PMMA. Serial sections of 30 μm were alternatively left unstained for fluorescence microscopy or stained with hematoxylin and eosin (HE). Unstained serial sections were used for qualitative analysis of bone ingrowth by fluorescence microscopy. HE-stained sections were analyzed to quantify bone ingrowth distance and cement penetration with interactive computer-controlled image analysis (AnalySIS; Soft Imaging System, Munster, Germany). For this purpose, a hemi-circle matching the inner surface of the porous titanium layer at the TiP-cement interface was drawn. The center of the corresponding circle resembled the center of the final semi-circular impactor. 12 lines were drawn from the center of the hemi-circle through the graft layer, at a mutual angle of 15 degrees. At the intersection of the line with the various composing layers of the reconstruction, the following parameters were quantified: (1) the thickness of the fibrous tissue interface between cement and the porous titanium layer; (2) the penetration depth of cement from the TiP-cement interface into the porous titanium layer; (3) the penetration depth

of ingrowing bone from the TiP-bone interface into the porous titanium layer; (4) the thickness of the porous titanium layer; and (5) the thickness of the fibrous tissue interface between the porous titanium layer and the surrounding host bone.

Wear analysis

Preoperatively, the surfaces of 4 new polyethylene cups and 4 new CoCr heads were inspected at different magnifications on a scanning electron microscope (Jeol 6310) equipped with an energy-dispersive X-ray detector (SEM-EDS). After retrieval of the acetabular reconstructions, the implanted cups and femoral heads were carefully detached without damaging the articular surfaces of the implants. Retrieved implants were visualized at the same magnifications as preoperatively. For comparison of damage, the articular surface of a retrieved cup from a previous goat study was used (acetabular impaction grafting with a mixture of bone particles and calcium phosphate particles) (Arts et al. 2005).

Systemic titanium analysis

Whole peripheral blood was collected at standardized time points (just before surgery and at 1, 7, 28, 56, and 105 days postoperatively) in metal-free vacuum containers (BD Vacutainer Trace Elements; BD Diagnostic Systems, Erembodegem, Belgium) and frozen at -40°C until analysis. Environmental and sampling contamination was avoided by the use of a laminar flow cabinet. All the disposable material used was tested for possible contamination in accordance with NF S 90 241, August 1990. Whole-blood samples were analyzed for cobalt content (control) and titanium content using an inductively coupled plasma mass dynamic reaction cell spectrometer (ICP-MS) with an AS-93 auto sampler (Perkin Elmer, Waltham, MA). 500 μL of whole blood was mixed with 500 μL 0.7% HNO_3, subsequently with 500 μl 70% HNO_3 and incubated overnight (12–14 h) at 80°C in a polypropylene 15-mL tube (Greiner Bio-One GmbH, Frickenhausen, Germany). The next day, 8.4 mL 0.7% HNO3 and 100 μL rhodium internal standard (CL01.11811.0100; Chem-lab, Zedelgem, Belgium) were added. Ultra-pure HNO3 was obtained after sub-boiling distillation in the laboratory of 70% HNO3 (Cat. 424000025; Perkin Elmer, Waltham, MA). Ultra-pure water was obtained from an Elgastat Maxima (Elga Ltd., Buckinghamshire, UK). Titanium (Ti 49.9448) was measured with ammonia (0.85 mL/min) as a reaction gas to minimize interference, and cobalt (Co 58.933) was measured without reaction gas. Calibration was performed by applying the standard addition method and by using NIST traceable Multi-element ICP QC standard solution (Cat. CL01.13774.0100; Chem-lab, Zedelgem, Belgium) at 3 concentrations for each element. The detection limits in the sample matrix were 0.08 μg/L for cobalt and 0.3 μg/L for titanium.

Statistics

We used univariate linear regression with the factors goat (random) and time (covariate) as independent variables to determine time dependence of systemic titanium and cobalt concentrations (with SPSS sorftware version 20.0). A log transformation was applied for whole-blood titanium and cobalt concentrations to meet the criteria of univariate analysis. Results are expressed as mean (SD) or median (range), as box plots (median, 25th and 75th percentiles, and extremes), or as parts per billion (ppb, i.e. μg/L).

Results

Animals, surgical technique, and retrieval of acetabular reconstructions

Surgical procedures were uneventful. TiPs showed excellent handling properties and deformability comparable to those of cancellous bone particles. 5.0–8.0 g TiPs were implanted in every goat. Macroscopically, no release of titanium microparticles was observed during or following impaction of the TiPs. The goats started loading of their operated leg within a few hours after surgery. 1 goat sustained a femoral fracture 3 days postoperatively and was killed. Thus, 9 goats were available for analysis. None of them had wound infections and all of them had symmetrical gait. Post-mortem radiographs and retrieval of the acetabular reconstructions showed that 1 goat (goat 9) had a dislocated femoral head without other abnormalities of the acetabular reconstruction. Furthermore, 1 reconstruction turned out to have failed at the cement-TiP interface: the cemented cup had detached with part of the cement layer from the still intact TiP-graft layer (goat 3). None of the reconstructions showed any signs of mechanical breakdown of the TiP-graft layer. In addition, there were no macroscopic signs of adverse histological events such as infection, synovitis, or metallosis.

Histomorphometry

Cement penetration was clearly visible at the non-decalcified cross sections of the acetabular cups: median 0.87 (0.35–1.87) mm (Figure 4A and Figure 5). Mean bone ingrowth distance was 2.79 (SD 0.76) mm and the mean thickness of the whole graft layer was 5.56 (SD 0.85) mm (Figure 5). In the HE-stained sections, the pink-stained bone could be seen in all specimens penetrating into the larger gaps between the individual TiPs (Figure 4B). The reconstruction that failed at the cement-TiP interface showed only very shallow cement penetration (0.35 mm) with direct contact between the cement and the porous titanium over only 43% of the interface (HE-stained sections). The 8 other reconstructions showed cement penetration at least twice as deep (median 0.87 (0.79–1.87) mm), with more direct contact between the cement and the porous titanium: 78% (SD 28). If present, the fibrous interface between cement and porous titanium was rather thin: 0.13 (0.02-0.77) mm. The mean fibrous tissue interface, measured over the whole cement-TiP interface distance, was 0.05 (SD 0.05) mm (Figure 5).
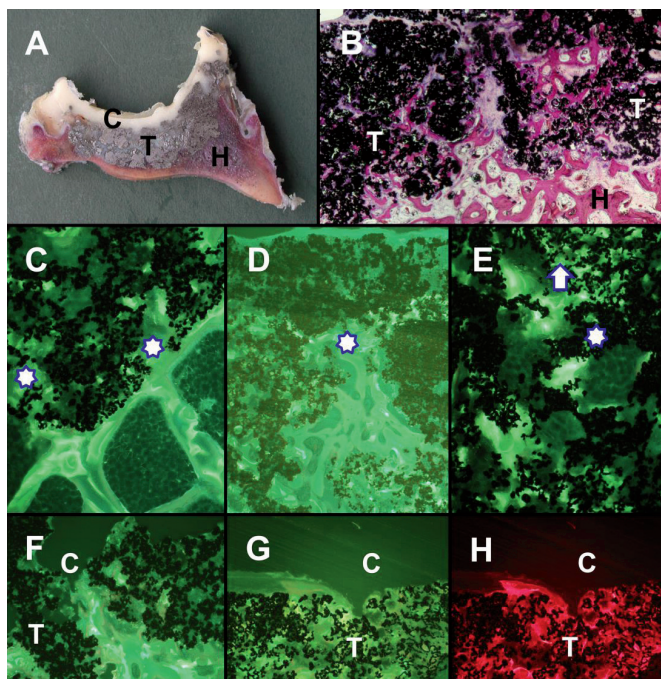
Figure 4. A. Cross section of reconstructed acetatubulum showing the host bone (H), TiP layer (T), and cement (C). B. HE-stained detail of the host bone (H)-TiP interface (T). C. Detail of host bone-TiP layer with calcein fluorescence. Asterisks indicate new bone. D. New bone formation in gap between 2 TiPs (asterisk). E Calcein fluorescence showing new bone formation in larger pores (asterisk) and smaller pores (white arrow) in a TiP. F. TiP (T)-cement (C) interface. G. and H. Same section and location but with different filter sets showing calcein and tetracycline fluorescence (panel G) and alizarin fluorescence (panel H). Notice thin soft tissue interface between TiP (T) and cement layer (C).
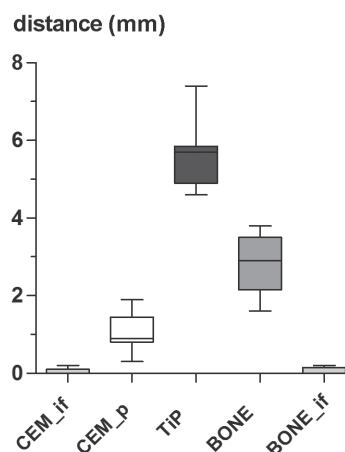

Figure 5. Histomorphometric quantification of the acetabular reconstruction: CEM_if: fibrous tissue layer between the bone cement and TiP; CEM_p: penetration depth of cement into the TiP layer; TiP: TiP-graft layer thickness; BONE: bone ingrowth depth in the TiP layer; BONE_if: thickness of the fibrous tissue layer between TiP and the surrounding bone.

At the interface between the host bone and the porous titanium layer, there was direct contact between bone and the porous titanium in all reconstructions (Figure 4C). This new bone was stained by fluorochromes (Figure 4C–H). Also, the smaller pores within the individual TiPs showed the presence of new bone (Figure 4E–H). Higher magnifications of both fluorescence and HE-stained preparations showed new bone in direct contact with the peripherally facing side of the TiP-graft layer at 94% (74–100) of the TiP-bone interface. The spots of fibrous interface between porous titanium and the surrounding bone were usually located at the inferomedial border of the reconstructive graft layer where fibrous tissue interposition occurred during impaction of the TiPs. Consequently, if present, the fibrous tissue interface was rather thick: mean 0.87 (SD 0.70) mm (Figure 4F–H). However, measured over the whole distance of the TiP-bone interface, the fibrous interface was only 0.01 (0.00–0.19) mm (Figure 5). Analysis of the TiP-graft layer showed that inter-particle pores (ranging from 50 to 1,000 μm) were larger than intra-particle pores (ranging from 10 to 100 μm). Analysis of the different fluorochrome labels showed penetration of new bone throughout the whole depth of the graft layer, both through the larger inter-particle pores and through the smaller-sized intra-particle pores, as

early as 4 weeks after implantation (Figure 4G). A gradual increase in the width and depth of the ingrowing bone was observed between 4 and 15 weeks after implantation. At many locations the bone, invading the porous titanium layer from peripherally, encountered the bone cement, penetrating the TiP-graft layer from centrally. There were no signs of titanium micro-particles or macrophage- or osteoclast-induced osteolysis (Figure 4).

Wear and systemic titanium concentrations

Before implantation, the articular surface of the cups showed machining marks; however, there were no signs of scratching or damage. The polished femoral head showed a smooth surface with only minor irregularities at high magnifications (Figure 6). After implantation, different wear patterns were observed. The 7 cups of the uneventful hips showed polishing wear with diminishment of machinery marks especially in the loaded area, and areas with some multidirectional, abrasive scratches in most cups. The femoral heads were unaffected apart from incidental, isolated abrasive unidirectional scratches (Figure 6). The dislocated hip and the reconstruction that failed at the cement-TiP interface showed wear comparable to that in the other cups. However, the femoral heads looked tarnished at the area of contact with the periphery of the reconstruction. Higher-magnification views revealed extensive, multidirectional deep scratches, which were probably the result of impingement against the rim of the stainless-steel mesh. Despite the wear of the femoral heads, no metallosis was observed and cobalt levels were comparable to those in the other goats.

In general, mean whole-blood cobalt concentrations were not time-dependent, measuring mean 1.40 (SD 0.37) ppb
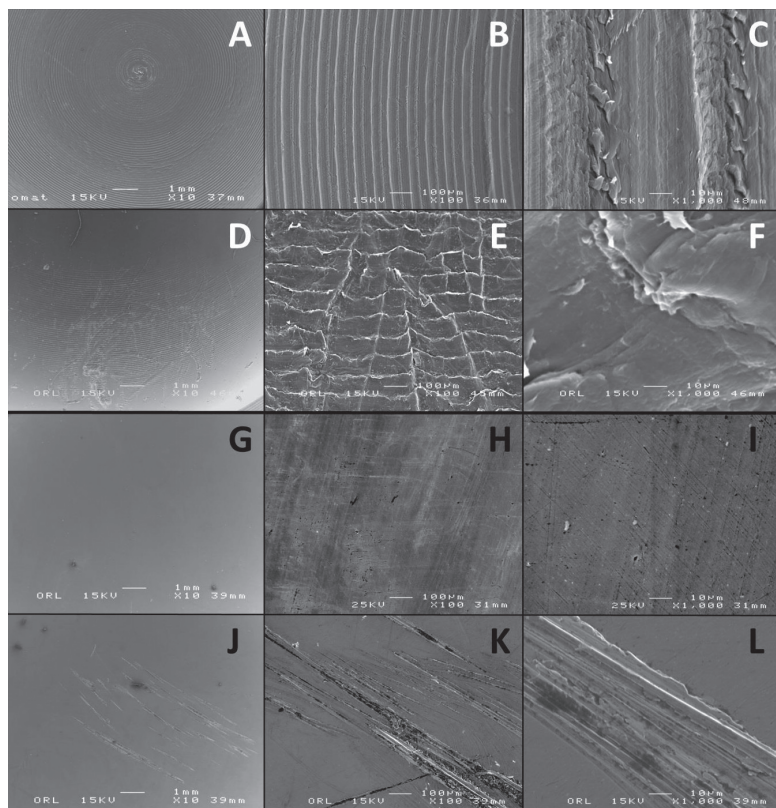
Figure 6. Representative views of the cup before (panels A–C) and after (D–F) implantation, and of the femoral head before (G–I) and after (J–L) implantation (scanning electron microscopy, with magnifications of 10× (left), 100× (middle), and 1,000× (right)).
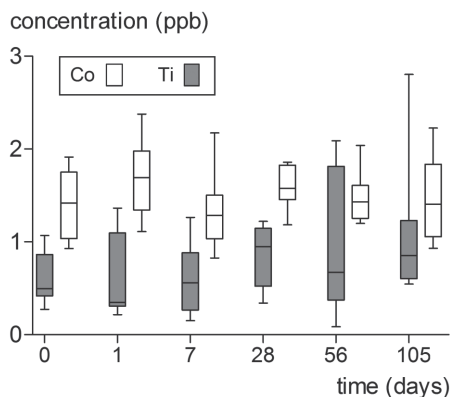

Figure 7. Time dependence of whole-blood concentration (parts per billion) of cobalt (Co) and titanium (Ti).

before operation and 1.46 (SD 0.46) ppb 15 weeks after operation (p = 0.8). In contrast to mean cobalt concentrations, median whole-blood titanium concentrations gradually increased throughout the experiment from 0.50 (0.27–1.07) ppb to 0.85 (0.55–2.80) ppb (p = 0.01) (Figure 7).

Discussion

This study focused on the suitability of porous titanium particles (TiPs) for the reconstruction of large acetabular bone defects with impaction grafting and a cemented cup. The model used was critical for evaluation of primary stability, due to several factors. Firstly, full weight bearing was allowed directly after surgery. Secondly, the goats were relatively old (retired milk goats, generally over 10 years of age), which would impair bone healing (Mehta et al. 2010). In addition, the femoral head was relatively large compared to the cup diameter, which causes increased shear stress within the reconstruction (Charnley 1970). We observed some differences compared to previous studies. Firstly, the size of the original bony acetabulum—and, as a result, the volume of the acetabular defect—was variable. Consequently, the volume of TiPs used varied between goats. This could lead to a difference in degree of impaction. However, the comparable bone growth distances in the goats would suggest that the variation in porosity of the graft layer was acceptable. Secondly, no titanium micro-particles were observed in high-magnification microscopic views, in contrast to previous studies where in vitro and ex vivo impaction were used (Walschot et al. 2010, 2011). The lack of observed micro-particles could reflect lower in vivo impaction forces. In addition, impaction in the relatively soft acetabular bone instead of in a metal chamber, and washout of particles by removed impaction fluid and pulse lavage rinsing, could have reduced the particle load.

Despite the challenges of the animal model used, the TiP-graft layer itself remained mechanically stable and became osseointegrated with the surrounding bone within 15 weeks. A fibrous tissue interface was almost absent. This is a striking difference compared to observations after bone impaction grafting and impaction grafting with ceramic particles, where the revascularization and re-ossification process is preceded by a fibrous transition zone, leaving a fibrous tissue interface between the bone graft and the opposing cement layer (Aspenberg et al. 1996, Arts et al. 2005). After incorporation of the graft layer, long-term stability becomes dependent on the fibrous tissue interface between cement and graft layer. This corresponds to the high incidence of radiolucent lines in the recently published clinical application of calcium phosphate particles (Whitehouse et al. 2013). From the viewpoint of homogeneity of mechanical characteristics, osseointegration would lead to less micro-motion and a more even stress distribution both within the reconstructive layer and at the interface between the prosthesis and the surrounding bone. In addition, a bony interface more efficiently seals against wear particles present in the joint fluid. From this viewpoint, coated

TiPs would be preferable to non-coated TiPs (Walschot et al. 2012). However, the hypothesis that a fibrous interface might be responsible for initiation of the loosening process, which is in agreement with the high rate of failure after acetabular bone impaction grafting in the same goat model (Schimmel et al. 1998, McEvoy et al. 2002, de Man et al. 2005), remains speculation as fibrous ingrowth into a graft layer also improves the primary stability (Tägil and Aspenberg 2001).

A possible cause for concern regarding any material used in impaction grafting is the release of micro-particles during impaction. Although the adverse response to micro-particles differs between materials, all micro-particles provoke an inflammatory and osteolytic reaction—especially micro-particles in the phagocytosable range (≤ 10 µm). The magnitude of the effect is proportional to the exposed dose of micro-particles (McEvoy et al. 2002, Warashina et al. 2003, O'Connor et al. 2004, Zysk et al. 2005, Lochner et al. 2011, Ding et al. 2012). Even micro-particles of calcium phosphate, a material that is considered to be osteoinductive, have been found to result in reduced osteoblast viability and fibrous incapsulation after implantation in the quadriceps of rats, with high macrophage and cellular counts in the fibrous capsule (Pioletti et al. 2002, Le Nihouannen et al. 2005, Yuan et al. 2006, Fellah et al. 2007). In order to assess the overall potential for induction of osteolysis, the size and total volume of bioceramic, titanium, and bone impaction debris particles were quantified in a previous in vitro study (Walschot et al. 2010). In summary, the volume of micro-particles ≤ 200 µm in diameter was about 400 times larger for bioceramic particles than for titanium particles, and about 30 times larger for bioceramic particles than for bone particles. These differences were even higher for the smaller-sized micro-particles (≤ 10 µm), where the volume of released micro-particles was 10,000 times larger for bioceramic particles than for titanium particles, and about 600 times larger for bioceramic particles, than for bone particles. These data should be kept in mind regarding the high incidence of radiolucent lines found in recently published medium-term results of clinical trials with impaction grafting with a mixture of bioceramic particles and bone particles (9 of 24 patients) and especially bioceramic particles alone (20 of 37 patients) (Whitehouse et al. 2013a, 2013b).

Besides a direct osteolytic effect of impaction debris, the impaction debris could generate additional micro-particles through third-body wear of the joint surface. This effect can be expected from both titanium debris and calcium phosphate debris and bone debris (Davidson et al. 1994), with a positive correlation between micro-particle size and the magnitude of the third body wear as well as between the exposed dose of micro-particle and the magnitude of the third body wear (Davidson et al. 1994, McNie et al. 2000). On the other hand, although the volume of micro-particles released is considerably larger during impaction of calcium phosphate particles than during impaction of calcium phosphate-coated titanium particles, titanium micro-particles are not degradable and

would probably be present in the joint fluid for longer than bioceramic micro-particles. Within the limitations of the time frame of our study, the extent and type of wear of the polyethylene cup and femoral heads appeared to be less or comparable to observations after impaction grafting with a mixture of bioceramic particles and bone particles (Arts et al. 2005). As observed with impaction grafting of calcium phosphate particles and bone particles, there were some abrasion marks at the surfaces of some cups and femoral heads. The wear was insufficient to clear all machining marks within 15 weeks.

An important finding in the present study was failure of 1 reconstruction at the cement-TiP interface. Microscopic analysis of this specimen showed the shallowest cement penetration (0.35 mm) of all reconstructions, with a fibrous tissue interface between the bone cement and the underlying porous titanium layer along the majority of the interface. As a result, there was insufficient direct interlocking of the bone cement with the surface of the TiP-graft layer. Cement penetration was limited in our study in general (0.87 (0.35–1.87) mm) compared to an in vitro study in synthetic acetabula (3.8 (SD 0.7) mm) with similar TiPs (Walschot et al. 2013). A bad fit of the pressurizer was noted with almost all reconstructed goat acetabula. As a result, the bone cement could not be pressurized adequately into the TiP-graft layer. Additionally, TiPs could be more susceptible to inferior pressurization due to the more micro-porous surface compared to bone particles.

Despite early osseointegration and limited micro-particle release and wear, there is still some concern about the long-term viability of the porous titanium reconstruction. Firstly, impaction grafting in primary hip replacement surgery—as in our study—is biologically and mechanically an advantageous situation compared to application in the revision setting of loosening after long standing osteolysis and sclerosis of the bone bed (Dohmae et al 1988). Secondly, the thickness of the graft layer was limited to 5.6 mm on average due to the size limitations of the animal model used. Thirdly, a short-term study has limitations with respect to prediction of long-term associated adverse events such as osteolysis and wear. The increase in titanium blood concentration is not only a known phenomenon in metallosis, but also in well-functioning titanium hip implants (Davidson et al. 1994, Kadoya et al. 1998, Catelas and Jacobs 2010). In our goat study, the titanium blood concentrations rose on a log-linear scale and would be expected to stabilize within concentrations as low as 1–2 ppb, which is comparable to reference values for humans without an implant (Jacobs et al. 1998). These values result from passive dissolution of titanium at the increasing contact area between the implant surface and invading host tissue, and are lower than observed in patients with a loose titanium hip prosthesis (approximately 8 ppb) (Jacobs et al. 1991, 1998). However, we cannot exclude the idea that non-absorbable titanium micro-particles cause accelerated wear of the joint surface during prolonged loading. The same holds for the potential of particle generation by fretting wear due to micro-motion

between individual TiPs within the graft layer. Thus, metal concentrations in blood should be monitored in a long-term clinical trial.

In conclusion, calcium phosphate-coated TiPs showed surgical handling properties comparable to those of cancellous bone particles, rapid osseointegration, and no indications of particle-induced adverse effects in a short-term loaded goat model. TiPs should be used with a proper cementing technique and a human pilot study should address the safety of application of TiPs in cemented impaction grafting in the long term, as well as their revisability.

Alffram P A, Bruce L, Bjursten L M, Urban R M, Andersson G B. Implantation of the femoral stem into a bed of titanium granules using vibration: a pilot study of a new method for prosthetic fixation in 5 patients followed for up to 15 years. Ups J Med Sci 2007; 112 (2): 183-9.

Aquarius R, Walschot L, Buma P, Schreurs B W, Verdonschot N. In vitro testing of femoral impaction grafting with porous titanium particles: a pilot study. Clin Orthop 2009; (467) (6): 1538-45.

Arts J J, Gardeniers J W, Welten M L, Verdonschot N, Schreurs B W, Buma P. No negative effects of bone impaction grafting with bone and ceramic mixtures. Clin Orthop 2005a; (438): 239-47.

Arts J J, Schreurs B W, Buma P, Verdonschot N. Cemented cup stability during lever-out testing after acetabular bone impaction grafting with bone graft substitutes mixes containing morselized cancellous bone and tricalcium phosphate-hydroxyapatite granules. Proc Inst MechEng [H] 2005b; 219 (4): 257-63

Aspenberg P, Tagil M, Kristensson C, Lidin S. Bone graft proteins influence osteoconduction. A titanium chamber study in rats. Acta Orthop Scand 1996; 67 (4): 377-82.

Bicanic G, Delimar D, Delimar M, Pecina M. Influence of the acetabular cup position on hip load during arthroplasty in hip dysplasia. Int Orthop 2009; 33 (2): 397-402.

Bolder S B, Verdonschot N, Schreurs B W, Buma P. Acetabular defect reconstruction with impacted morsellized bone grafts or TCP/HA particles. A study on the mechanical stability of cemented cups in an artificial acetabulum model. Biomaterials 2002; 23 (3): 659-66.

Catelas I, Jacobs J J. Biologic activity of wear particles. Instr Course Lect 2010; 59: 3-16.

Charnley J. Total hip replacement by low-friction arthroplasty. Clin Orthop 1970; (72): 7-21.

Davidson J A, Poggie R A, Mishra A K. Abrasive wear of ceramic, metal, and UHMWPE bearing surfaces from third-body bone, PMMA bone cement, and titanium debris. Biomed Mater Eng 1994; 4 (3): 213-29.

de Man F H, Tigchelaar W, Marti R K, Van Noorden C J, Van der Vis H M. Effects of mechanical compression of a fibrous tissue interface on bone with or without high-density polyethylene particles in a rabbit model of prosthetic loosening. J Bone Joint Surg (Am) 2005; 87 (7): 1522-33.

Ding Y, Qin C Q, Fu Y R, Xu J, Huang D S. In vitro comparison of the biological activity of alumina ceramic and titanium particles associated with aseptic loosening. Biomed Mater 2012; 7 (4): 045019.

Dohmae Y, Bechtold J E, Sherman R E, Puno R M, Gustilo R B. Reduction in cement-bone interface shear strength between primary and revision arthroplasty. Clin Orthop 1988; (236): 214-20.

Fellah B H, Josselin N, Chappard D, Weiss P, Layrolle P. Inflammatory reaction in rats muscle after implantation of biphasic calcium phosphate micro particles. J Mater Sci Mater Med 2007; 1 8(2): 287-94.

Flecher X, Parratte S, Brassart N, Aubaniac J M, Argenson J N. Evaluation of the hip center in total hip arthroplasty for old developmental dysplasia. J Arthroplasty 2008; 23 (8): 1189-96.

Garcia-Cimbrelo E, Marti-Gonzalez J C. Circular external fixation in tibial nonunions. Clin Orthop 2004; (419): 65-70.

Gie G A, Linder L, Ling R S, Simon J P, Slooff T J, Timperley A J. Impacted cancellous allografts and cement for revision total hip arthroplasty. J Bone Joint Surg (Br) 1993; 75 (1): 14-21.

Jacobs J J, Skipor A K, Black J, Urban R, Galante J O. Release and excretion of metal in patients who have a total hip-replacement component made of titanium-base alloy. J Bone Joint Surg (Am) 1991; 73 (10): 1475-86.

Jacobs J J, Skipor A K, Patterson L M, Hallab N J, Paprosky W G, Black J, et al. Metal release in patients who have had a primary total hip arthroplasty. A prospective, controlled, longitudinal study. J Bone Joint Surg (Am) 1998; 80 (10): 1447-58.

Kadoya Y, Kobayashi A, Ohashi H. Wear and osteolysis in total joint replacements. Acta Orthop Scand (Suppl 278)1998: 1-16.

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 (1): 39-48.

Le Nihouannen D, Daculsi G, Saffarzadeh A, Gauthier O, Delplace S, Pilet P, Layrolle P. Ectopic bone formation by microporous calcium phosphate ceramic particles in sheep muscles. Bone 2005; 36 (6): 1086-93.

Lee J M, Nam H T. Acetabular revision total hip arthroplasty using an impacted morselized allograft and a cementless cup: minimum 10-year follow-up. J Arthroplasty 2011; 26 (7): 1057-60.

Leeuwenburgh S, Layrolle P, Barrere F, de Bruijn J, Schoonman J, van Blitterswijk C A, et al. Osteoclastic resorption of biomimetic calcium phosphate coatings in vitro. J Biomed Mater Res 2001; 56 (2): 208-15.

Lochner K, Fritsche A, Jonitz A, Hansmann D, Mueller P, Mueller-Hilke B, Bader R. The potential role of human osteoblasts for periprosthetic osteolysis following exposure to wear particles. Int J Mol Med 2011; 28 (6): 1055-63.

McEvoy A, Jeyam M, Ferrier G, Evans C E, Andrew J G. Synergistic effect of particles and cyclic pressure on cytokine production in human monocyte/macrophages: proposed role in periprosthetic osteolysis. Bone 2002; 30 (1): 171-7.

McNie C M, Barton D C, Fisher J, Stone M H. Modeling of damage to articulating surfaces by third body particles in total joint replacements. J Mater Sci Mater Med 2000; 11 (9): 569-78.

Mehta M, Strube P, Peters A, Perka C, Hutmacher D, Fratzl P, et al. Influences of age and mechanical stability on volume, microstructure, and mineralization of the fracture callus during bone healing: is osteoclast activity the key to age-related impaired healing? Bone 2010; 47 (2): 219-28.

O'Connor D T, Choi M G, Kwon S Y, Paul Sung K L. New insight into the mechanism of hip prosthesis loosening: effect of titanium debris size on osteoblast function. J Orthop Res 2004; 22 (2): 229-36.

Ornstein E, Franzen H, Johnsson R, Stefansdottir A, Sundberg M, Tagil M. Five-year follow-up of socket movements and loosening after revision with impacted morselized allograft bone and cement: a radiostereometric and radiographic analysis. J Arthroplasty 2006; 21 (7): 975-84.

Paprosky W G, O'Rourke M, Sporer S M. The treatment of acetabular bone defects with an associated pelvic discontinuity. Clin Orthop 2005; (441): 216-20.

Pioletti D P, Takei H, Lin T, Van Landuyt P, Ma Q J, Kwon S Y, Sung K L. The effects of calcium phosphate cement particles on osteoblast functions. Biomaterials 2000; 21 (11): 1103-14.

Schimmel J W, Buma P, Versleyen D, Huiskes R, Slooff T J. Acetabular reconstruction with impacted morselized cancellous allografts in cemented hip arthroplasty: a histological and biomechanical study on the goat. J Arthroplasty 1998; 13 (4): 438-48.

Swedish Hip Arthroplasty Register (Internet]). Garellick G, Kärrholm J, Rogmark C, Herberts P. Annual Report 2008. Available from: https://www.jru.orthop.gu.se/ October 2009.

Tägil M, Aspenberg P. Fibrous tissue armoring increases the mechanical strength of an impacted bone graft. Acta Orthop Scand 2001; 72 (1): 78-82.

Turner T M, Urban R M, Hall D J, Andersson G B. Bone ingrowth through porous titanium granulate around a femoral stem: histological assessment in a six-month canine hemiarthroplasty model. Ups J Med Sci 2007; 112 (2): 191-7.

van der Donk S, Buma P, Verdonschot N, Schreurs B W. Effect of load on the early incorporation of impacted morsellized allografts. Biomaterials 2002; 23 (1): 297-303.

van Haaren E H, Smit T H, Phipps K, Wuisman P I, Blunn G, Heyligers I C. Tricalcium-phosphate and hydroxyapatite bone-graft extender for use in impaction grafting revision surgery. An in vitro study on human femora. J Bone Joint Surg (Br) 2005; 87: 267–71.

van Haaren E H, Heyligers I C, Alexander F G, Wuisman P I. High rate of failure of impaction grafting in large acetabular defects. J Bone Joint Surg (Br) 2007; 89 (3): 296-300.

Walschot L H, Schreurs B W, Buma P, Verdonschot N. Impactability and time-dependent mechanical properties of porous titanium particles for application in impaction grafting. J Biomed Mater Res Part B, Applied biomaterials 2010; 95 (1): 131-40.

Walschot L H, Schreurs B W, Verdonschot N, Buma P. The effect of impaction and a bioceramic coating on bone ingrowth in porous titanium particles. Acta Orthop 2011; 82 (3): 372-7.

Walschot L H, Aquarius R, Schreurs B W, Verdonschot N, Buma P. Osteoconduction of impacted porous titanium particles with a calcium-phosphate coating is comparable to osteoconduction of impacted allograft bone particles: in vivo study in a nonloaded goat model. J Biomed Mater Res Part B, Applied biomaterials 2012; 100 (6): 1483-9.

Walschot L H, Aquarius R, Schreurs B W, Buma P, Verdonschot N. Better primary stability with porous titanium particles than with bone particles in cemented impaction grafting: An in vitro study in synthetic acetabula. J Biomed Mater Res Part B, Applied biomaterials 2013; 101 (7): 1243-50.

Warashina H, Sakano S, Kitamura S, Yamauchi K I, Yamaguchi J, Ishiguro N, Hasegawa Y. Biological reaction to alumina, zirconia, titanium and polyethylene particles implanted onto murine calvaria. Biomaterials 2003; 24 (21): 3655-61.

Whitehouse M R, Dacombe P J, Webb J C, Blom A W. Impaction grafting of the acetabulum with ceramic bone graft substitute: high survivorship in 43 patients with a mean follow-up period of 4 years. Acta Orthop 2013a; 84 (4): 371-6.

Whitehouse M R, Dacombe P J, Webb J C, Blom A W. Impaction grafting of the acetabulum with ceramic bone graft substitute mixed with femoral head allograft: high survivorship in 43 patients with a median follow-up of 7 years: a follow-up report. Acta Orthop 2013b; 84 (4): 365-70.

Yuan H, van Blitterswijk C A, de Groot K, de Bruijn J D. A comparison of bone formation in biphasic calcium phosphate (BCP) and hydroxyapatite (HA) implanted in muscle and bone of dogs at different time periods. J Biomed Mater Res A 2006; 78 (1): 139-47.

Zysk S P, Gebhard H H, Kalteis T, Schmitt-Sody M, Jansson V, Messmer K, Veihelmann A. Particles of all sizes provoke inflammatory responses in vivo. Clin Orthop 2005; (433): 258-64.