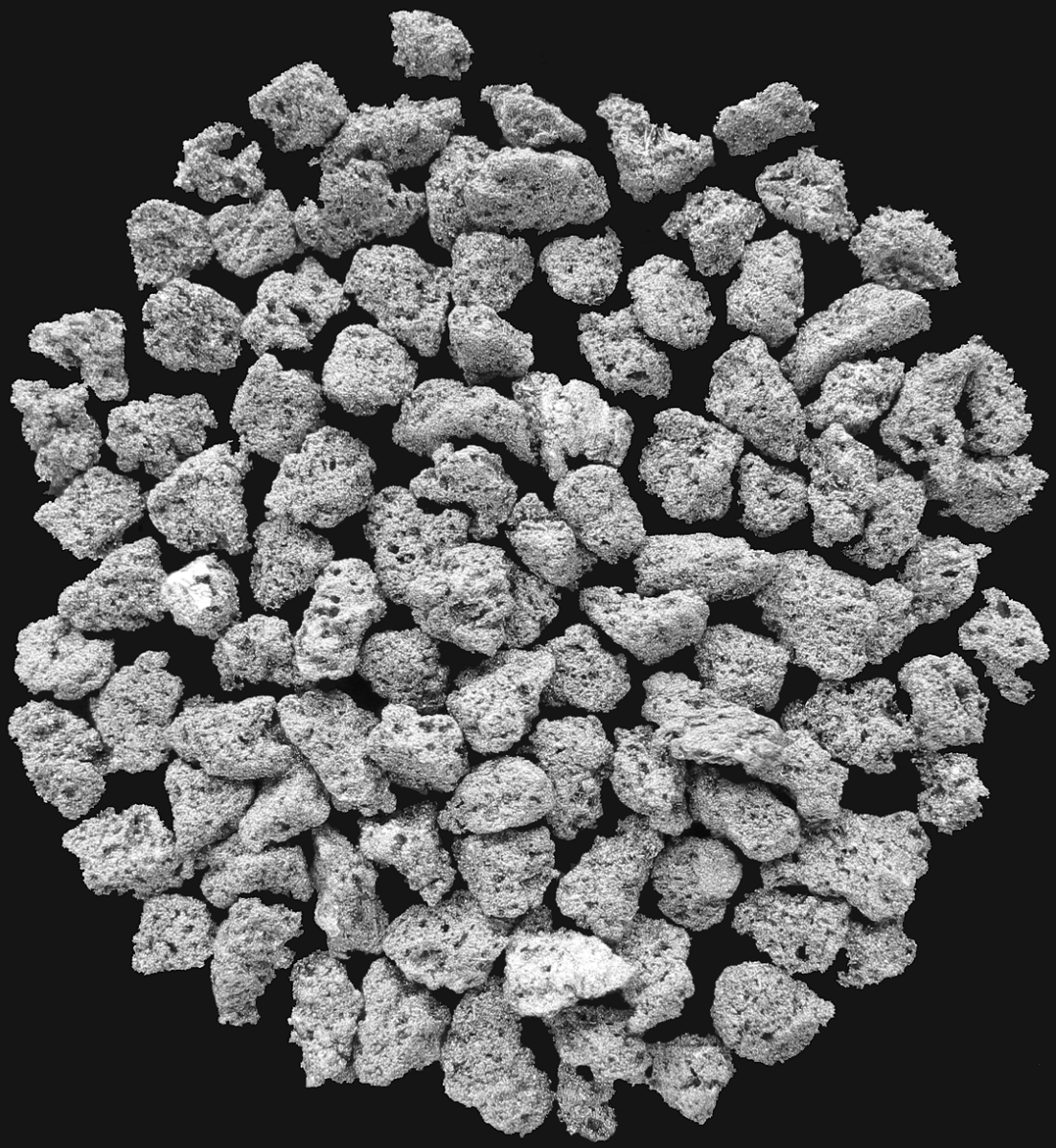
POROUS TITANIUM PARTICLES AS A FULL BONE SUBSTITUTE FOR DEFECT RECONSTRUCTION



IN HIP JOINT REPLACEMENT SURGERY

L.H.B. Walschot

POROUS TITANIUM PARTICLES AS A FULL BONE SUBSTITUTE FOR DEFECT RECONSTRUCTION IN HIP JOINT REPLACEMENT SURGERY

Lucas Hubert Bernard Walschot

COLOFON

The data for this thesis were collected at the Orthopaedic Research Laboratory, Department of Orthopaedics, Radboud University Nijmegen, Nijmegen, The Netherlands.

I gratefully acknowledge the financial support for publication of this thesis provided by the Anna Fonds/NOREF Foundation (Leiden, The Netherlands), the Dutch Orthopaedic Association (Nederlandse Orthopaedische Vereniging, 's-Hertogenbosch, the Netherlands), and the RMS Foundation (Bettlach, Switzerland).

Testing • Research • Consulting

www.rms-foundation.ch

NEDERLANDSE
ORTHOPAEDISCHE
VERENIGING | NOV

ISBN

978-94-6108-580-1

NUR

883 Medische en socio-medische wetenschappen

PRINTING

Gildeprint - Enschede

GRAPHIC DESIGN

www.gildeprint.nl

POROUS TITANIUM PARTICLES AS A FULL BONE SUBSTITUTE FOR DEFECT RECONSTRUCTION IN HIP JOINT REPLACEMENT SURGERY

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op woensdag 5 februari 2014
om 14.30 uur precies

door

L.H.B. Walschot
geboren op 10 oktober 1975 te Roermond

Promotoren

Prof. dr. P. Buma

Prof. dr. ir. N.N.J. Verdonschot

Copromotor

Dr. B. W. Schreurs

Manuscriptcommissie

Prof. dr. J.A. Jansen

Prof. dr. I.C. Heyligers (UM)

Prof. dr. ir. H. H. Weinans (UU)

CONTENTS

CONTRIBUTING PUBLICATIONS

1. Aquarius R, Walschot LH, Buma P, Schreurs BW, Verdonschot N. In Vitro testing of Femoral Impaction Grafting with Porous Titanium Particles: a pilot study. Clin Orthop Relat Res 2009;467(6):1538-45.
2. Walschot LH, Schreurs BW, Buma P, Verdonschot N. Impactability and time-dependent mechanical properties of porous titanium particles for application in impaction grafting. J Biomed Mater Res B Appl Biomater 2010;95(1):131-40.
3. Walschot LH, Schreurs BW, 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.
4. Walschot LH, Aquarius R, Schreurs BW, 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 non-loaded goat model. J Biomed Mater Res B Appl Biomater 2012;100(6):1483-9.
5. Walschot LH, Aquarius R, Schreurs BW, 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 B Appl Biomater 2013;101(7):1243-50.
6. Walschot LH, Aquarius R, Verdonschot N, Buma P, Schreurs BW. Porous titanium particles for acetabular reconstruction in hip arthroplasty show extensive bony armoring after fifteen weeks. A loaded in vivo study in ten goats. Submitted to Acta Orthopaedica.

CHAPTER ONE

GENERAL INTRODUCTION

Epidemiology of hip joint arthritis

Painless and mobile joints are a prerequisite to maintain our level of functioning and daily joy in life. The joint surface is covered with a thin layer of lubricating cartilage. Cartilage can be damaged both mechanically (by loading and trauma) and biologically (by sterile inflammatory conditions and infection). Unfortunately cartilage has a poor intrinsic capacity for healing. Instead of repair, degeneration occurs. Degeneration and loss of the cartilage layer are accompanied by reactive periarticular bone remodelling and contracture of the surrounding joint capsule [1]. This process is chronic and progressive and is called arthritis (from Greek *arthros-* = joint *–itis* = inflammation). Arthritis is divided on basis of aetiology into primary arthritis, also called osteoarthritis (OA, 80% of hip joint arthritis, no other known cause than polygenetic susceptibility), and secondary arthritis (20% of hip joint arthritis, due to auto immune disease like rheumatoid arthritis, traumatic damage to the joint, childhood hip joint disease, etcetera) [2,3]. Both OA as well as secondary arthritis will lead to degradation of articular cartilage and an impaired joint function. The impact of OA on the quality of life of the patient is of particular importance [4-6]. Musculoskeletal disorders are associated with lower physical activity and more pain in the affected tissues than for instance cardiovascular conditions and chronic respiratory diseases [4]. The direct and indirect economic burden for society is rising and accounts for 0.5% to 2.5% of the gross national product of western countries [4,7,8]. One out of eight persons will suffer from OA. Besides female gender, increasing age and over-weight are risk factors becoming more and more important [1,4-10]. More specifically one out of twenty people will suffer from arthritis of the hip and this disease is associated with a considerable decrease in physical and mental function [9,10].

History and current treatment concepts

Non-operative management of hip arthritis is the initial treatment of choice and prevents or delays the need for a hip replacement [1]. Optimal non-operative treatment requires a combination of non-pharmacological and pharmacological modalities [1,11,12]. In a minority of people, non-operative management fails and operative management (total hip arthroplasty (THA): *arthros* = joint, *plassein* = to shape) is indicated.

The natural hip joint consists of a ball (femoral head, upper part of the femur) articulating in a socket (acetabulum, part of the pelvis). Primary THA replaces the natural hip joint

by a total hip prosthesis: the acetabulum is replaced by an artificial socket (cup) and the femoral head is replaced by an artificial ball (head) on top of a metal stem (stem) inserted in the upper part of the femur. The first start was made by Themistocles Glück in 1891 in Germany, using ivory to replace the femoral head. In the 1960's a major breakthrough was achieved by Sir John Charnley who introduced ultra-high molecular weight polyethylene (PE) cup with a metal femoral head and stem (cobalt-chromium alloy) and fixation of the cup and the stem with polymethylmethacrylate (PMMA) bone cement [13]. The concept of the cemented THA proved to be successful and still remains relatively unchanged. Meanwhile, direct fixation of the cup and the stem to the bone, without interposition of cement, has been introduced. Recent data show that in terms of long-term survival, cemented THA's still perform better than uncemented THA's [14].

Incidence of primary and revision THA

Replacement of a symptomatic arthritic hip joint (primary THA) is highly cost effective and might be more cost effective than non-operative treatment modalities for arthritis. [11,12,15]. Compared to generally accepted health care modalities like breast cancer screening (€ 24.000 - € 36.000 / QUALY), cardial revascularization with a drug eluting stent (€ 38.000 - 60.000 / QUALY) or renal transplantation (€ 54.000 - € 127.000 / QUALY), primary THA (€ 6.710 / QUALY) is considerably more cost effective. THA substantially increases physical functioning, body pain and also psychosocial functioning. Although primary THA does not restore the level of pain and physical functioning to values of people without arthritis of the hip, patient satisfaction is high (>90%) and durable (>15 years) [16-18]. Annually as many as one million hip replacements are performed [19]. This number has increased 10% between 2005 and 2008 and will continue to increase in the coming decades due to demographic factors. Unfortunately a primary THA has a limited life span. Failure of a THA usually necessitates replacement of the failed THA (revision THA). The most frequent mode of failure is loosening and resorption of bone (osteolysis) (70-75%) followed by instability (8%), infection (8%) and fracture of the surrounding bone (6%) [3]. Current 10-year survival rates of THA's in Finland, Norway, Sweden and England range from 80-95% and due to the age at primary surgery (65-70 years) most THA's will out-live their owner [3,14]. However, the 10-year survival rates of THA's in young patients (aged below 55-60 years at primary surgery) are only 72-86%. As a result it is this young

patient who is at risk for one or multiple revision procedures [20]. The number of revision THA's amounted to 100.000 in the US and Europe in 2005 and is expected to rise linearly with the number of primary THA's [3,19,21-23]. Although revision THA is a technically elaborate operation compared to a primary THA, it is still very cost effective [24-26].

Bone stock loss

A major challenge in both primary and revision THA is dealing with bone deficiency. The presence of adequate surrounding bone is a prerequisite for anatomical positioning of the prosthesis (which maximizes function and survival), stable fixation of the prosthesis and sufficient bone stock to carry out a possible future revision THA [27-29]. Due to the secondary nature of arthritis in younger patients (rheumatoid arthritis, osteonecrosis, congenital hip dysplasia) it is often this patient who presents him/herself with bone defects of the acetabulum at primary THA [28]. Bone defects during revision THA are encountered at the acetabular side (around the cup), on the femoral side (around the stem) or both [30]. These defects usually result from wear-particle induced osteolysis and abrasion of bone by the loosened prosthesis [31]. Further loss of bone stock occurs during removal of the failed prosthesis which is intrinsic to the nature of the revision operation.

Bone defects of the acetabulum

The goals of reconstructing the acetabulum are to relocate the hip joint to the anatomic hip centre with adequate fixation to obtain long-term survival and restoration of bone stock to enable a future revision. The geometry of the defect determines which technique can be used for acetabular bone defects. The classification of the American Academy of Orthopaedic Surgeons (AAOS) divides the defects in five types (I–V): segmental (I), cavitary (II), combined (III), pelvic discontinuity (IV) and arthrodesis (V) (Figure 1) [32].

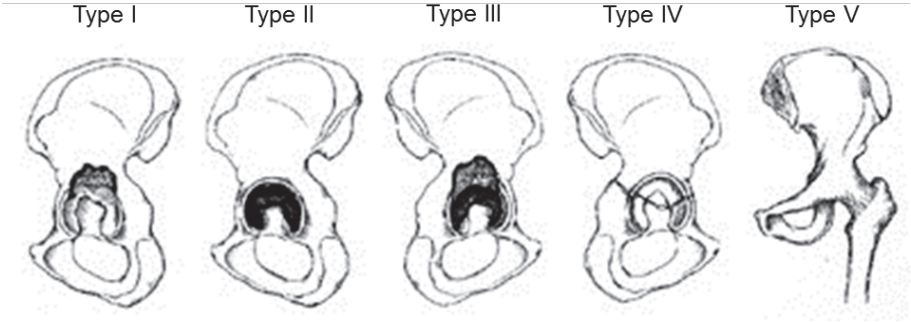


Figure 1 *Classification of acetabular bone defects according to D'Antonio et al.*

Segmental defects, cavitary defects and minor combined defects (>50% of the cup in contact with host bone, IIIA) can be reconstructed with a cemented or uncemented cup. Good results have been published for both techniques except for filling of a defect with bone cement. This technique should only be used in patients with a short life expectancy [33]. Large cups do not restore bone stock and often result in a suboptimal position of the hip joint which is associated with decreased function and survival [27,29,34]. Of all these methods only impaction grafting has proven to combine all desired features (adequate position of the joint, good long-term survival, bone stock repair) (Figure 2) [28,30]. Larger combined defects (<50% of the cup in contact with host bone, IIIB) and pelvic discontinuity (IV) have been treated by a structural allograft with an uncemented cup, a structural graft with a cage, a trabecular metal augment with an uncemented cup, custom made tri-flanged cups and by bone impaction grafting with a cemented cup and an uncemented cup [35-46]. A high incidence of failure was seen with structural allografts in combination with an uncemented cup after which the cage, the triflange cup and trabecular metal cups and augments were introduced [35-37,43,45] Alternatively a cage can be used together with a massive acetabular allograft [41,42,44]. So far no long-term data are available and mid-term results are highly variable. The technique of bone impaction grafting can also be used to reconstruct these large defects. However, in this situation the technique is more demanding and results in a thick graft layer. Although good long-term results have been reported by several authors in type IIIB defects, a high failure rate in type IIIB, and type IV defects was reported by others [38,40,46,47]. More extensive impaction grafting reconstruction have thicker graft layers and probably higher

postoperative migration rates. Although good results have been reported with cemented acetabular impaction grafting despite migration of > 1 mm within the first postoperative year, radiographic loosening at five years follow-up was only observed in cups with high rates of initial migration and rotation as measured by radiostereometric analysis (RSA) [48,49]. Type V defects (arthrodesis) implicate no actual deficiency of acetabular bone stock, although the true acetabulum may be difficult to locate.

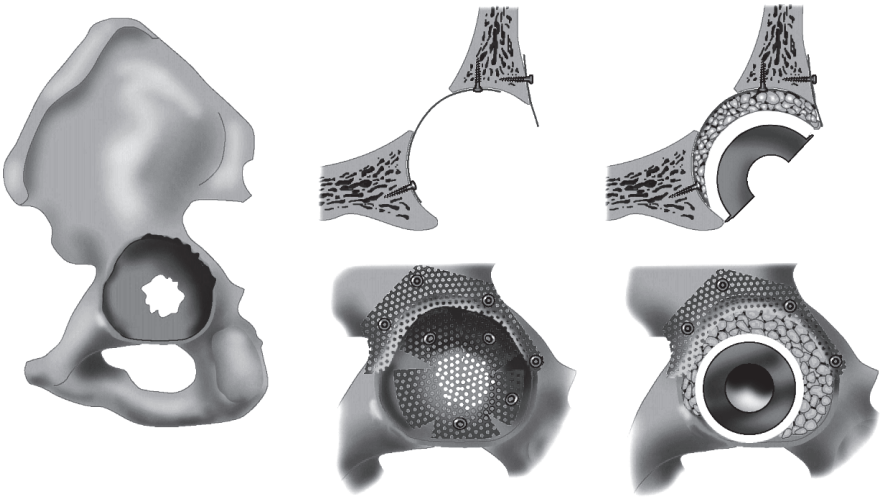


Figure 2 *Acetabular Bone Impaction Grafting.*

Bone defects of the femur

Femoral bone stock is deficient to some degree in most revisions and may result from 1) osteolysis caused by loosening, wear of infection; 2) perforation or creation of a window during removal of the previous stem or other implant; 3) stress shielding (reduction in bone density (osteopenia) as a result of removal of normal stress from the bone) by a previous (distally fixating) implant and 4) pre-existing osteoporosis with thin femoral cortices. Reconstruction can further be complicated by a femoral deformity or fracture. The treatment of femoral deficiencies has many parallels to the treatment of acetabular deficiencies, including the goals (obtain adequate fixation for long-term stability and restore bone stock to enable a future revision) and the availability of classification.

Femoral defects are classified into six types according to the AAOS. This classification was simplified by Valle and Paprosky [50]. Four types of defects (I-IV) fit an algorithmic approach for femoral reconstruction: minimal loss of metaphyseal cancellous bone and an intact diaphysis (I), extensive loss of metaphyseal bone and an intact diaphysis (II), severely damaged, non-supportive metaphysis and proximal diaphysis with > 4 cm diaphysis available for distal fixation (IIIA), severely damaged and non-supportive metaphysis with < 4 cm diaphysis available for distal fixation (IIIB), extensive metaphyseal and diaphyseal damage with a widened canal and a non-supportive diaphysis (IV). Cemented or uncemented stems can be used for type I defects. Type II defects have little remaining cancellous bone and direct cementing with a standard stem length is therefore unreliable. A long, distally fixating uncemented stem can be chosen, a long cemented stem or a cemented stem with bone impaction grafting [51-54]. Impaction grafting should not be combined with an uncemented stem [54]. Corresponding to management of peri-prosthetic fractures, a type IIIA defect can be bridged by a long, distally fixated, extensive coated uncemented stem [54,55]. Cemented reconstruction with impaction grafting is used for both type IIIA and IIIB defects (Figure 3). Most frequently reported complications in earlier reports are high early subsidence rates, high failure rates and peri-prosthetic fractures with a standard length stem [56,57]. Biomechanical analysis shows that in peri-prosthetic fracture management, the stem should bypass the defect or the fracture at least over a length equal to or more than 2 times the diameter of the diaphysis just distal to the defect or fracture [58]. Correspondingly, long stems that bypass a cortical defect or distal osteolysis show better survival than standard length stems. If a long stem is not available, the cortical defect should be reinforced with a strut graft and cables. In extensive metaphyseal defects it is advocated to reconstruct the calcar with a mesh or a strut allograft to increase rotational stability. Together with a long stem, this results in a stable and durable reconstruction with 10-year survival rates >90% in multi-centre application [59-64]. Type IV defects are beyond repair by impaction grafting and require bridging by a modular prosthesis or tumour prosthesis. Alternatively, a distally fixated prosthesis can be used with a segmental allograft to replace the absent proximal femur [65].

Figure 3 *Femoral Bone Impaction Grafting.*

Impaction grafting with autograft or allograft bone and basics of the incorporation process

If bone grafts are used for small reconstructions, often the patient's own bone is recycled (autograft bone, autografts). If autograft particles can not be obtained by nibbling or milling of the femoral head like during primary surgery, the iliac crest has to be used with additional morbidity and pain for the patient which can be worse than the pain from the hip operation itself (donor site morbidity) [66]. If large bone volumes are needed (>50ml) or if the femoral head is absent after removal during previous surgery, allografts have to be used. However, application of allograft bone is also associated with drawbacks like the need for storage equipment, limited conservation period, shortage of donor bone, risk of pathogen transmission and religious objections [67-69]. Large defects can be reconstructed by fixation of a large piece of allograft bone (structural allograft or bulk allograft) or by impaction of smaller cancellous or cortico-cancellous bone particles (impaction grafting). This thesis focuses on the impaction grafting technique as it has proven to combine all three desired goals in hip reconstruction surgery: reconstruction of the original anatomy, durable fixation of the prosthesis and creation of new and vital bone stock which makes it possible to fixate a new prosthesis in the future in case of failure. Instead of filling

the defect with a larger prosthesis, the original bone stock is reconstructed with bone grafts. Graft remodeling after the operation involves a complicated sequence of biological events which has only been partly characterized to date. In general, graft incorporation of autografts is faster and more complete than of allografts, incorporation in the acetabulum is more complete than in the femur and the incorporation process is stimulated by loading [70-76]. More in detail, biopsy and retrieval studies show the following: minimum bone formation may be initiated 1 month after implantation, after 8–11 month, the osteoid/ bone layer might have a thickness of 3–5 mm and after 2 years there is mainly bone with hematopoietic marrow [70-73,75]. The whole porous reconstructive layer of impacted bone grafts will thus be slowly replaced with living bone as opposed to massive grafts which show only superficial incorporation. At some locations - and probably those that do not carry load - the graft will be resorbed and may not be replaced with living bone. Ullmark et al used positron emission tomography to study bone metabolism and blood flow in the impacted area around cemented femoral stems up to 1 year after the operation. As early as eight days after the operation, blood flow and bone formation were demonstrated adjacent to the allograft. As early as one year after the operation, bone formation was found close to the cement mantle [77]. Patients are not allowed full weight bearing during the first six to twelve weeks to enable vascularization of the graft layer as primary stability of the bone graft layer is still a reason of concern.

Why porous titanium particles as a bone substitute material in impaction grafting?

The potential advantage of synthetic bone substitute materials for restoration of bone defects in general are reflected by the growing number of publications about developments on this topic. The question of ‘’what is a suitable full bone substitute biomaterial for impaction grafting’’ is not difficult to answer. The material should be:

1. Deformable (ductile).
2. Available in morsels of the desired size.
3. Resistant to loading and shear stress.
4. Porous to allow fibrous or bony reinforcement for long-term stability.
5. Biocompatible.
6. Iso-elasticity compared to bone.
7. Corrosion resistance.
8. Unlimited available at an affordable price.

In short, deformability and availability in morsels of the desired size enable adaption to different defect geometries. Good resistance to compressive, tensile and shear stresses are necessary for primary stability and allows early postoperative load bearing [78]. Porosity, biocompatibility and iso-elasticity enable ingrowth of host tissue which is beneficial for long-term stability of the reconstruction. Finally, corrosion resistance prevents weakening of the reconstruction and local and/or systemic undesired side effects of increased concentrations of the degraded biomaterial.

Grossly speaking, three different materials have been topic of interest in the literature:

1. Resorbable: calcium containing minerals
2. Resorbable: polymers
3. Non resorbable: metals

Although biodegradable polymers are associated with foreign body reactions, all three materials have proven to be biocompatible and osteoconductive and from this point of view there is no clear ''winner'' [79,80]. Pure calcium phosphate containing materials exhibit inferior handling characteristics and have to be mixed with softer bone particles for application in impaction grafting [81-85]. The most important drawback of biodegradable polymers is also inferior mechanical characteristics: biodegradable polymers possess only very low compressive strength [86]. Consequently, so far bioresorbable polymers have been applied predominantly in non loaded conditions or for fracture fixation and are not suitable for application in a loaded joint reconstruction. In contrast to polymers and calcium containing bioceramics, metals can combine the three desirable mechanical properties important for application in impaction grafting: ductility, resistance to compressive stress and resistance to tensile stress (Table 1) [87-100]. Metals applied in orthopaedic surgery can be divided into four groups.

A. Stainless steel (FeCrNi)
B. Cobalt-Chromium-Molybdenum (CoCrMo) alloys
C. Tantalum (Ta)
D. Commercially pure titanium (cp Ti) and titanium alloys

Each metal group has it's specific combination of properties. Stainless steel combines excellent ductility with a low price. However, due to its Ni and Cr content the biocompatibility is inferior to Ti. As a result Ni free stainless steel has been developed. CoCr implants are among the lest ductile metals and are very stiff. This results in stress

shielding. Ta is very corrosion resistant and less stiff than stainless steel and CoCr but stiffer than Ti and ten times more expensive.

MECHANICAL PROPERTIES OF DIFFERENT BIOMATERIALS				
	density (g/cm^3)	stiffness (GPa)	ultimate tensile strength (MPa)	elongation at fracture (%)
METALS				
CrNiFe	7.8	200	450-1000	40
CoCrMo	8.5	200-230	600-1800	8
cp Ti	4.5	105	800	22
Ti (α+β)	4.5	110	950	8
Ti (β)	4.5	60	1000	10
Ta	16.6	190	285	30
CERAMICS				
Al_2O_3	4.0	350	600	<1
ZrO_2	6.0	200	900	<1
HA	3.2	70-120	40-100	<1
RESORBABLE POLYMER				
PLA	1.3	1.2-3	30-50	2-6
PCL	1.0	0.4	15	50-80
NONRESORBABLE POLYMER				
PMMA	1.2	2	50[a]	1-2
UHMWPE	1.0	0.8-2.5	40	200-350
BONE				
cortical	1.9	17	130	1
cancellous	0.7	0.1-0.5	50	4

Table 1 *Density, stiffness, strength and ductility of different biomaterials.*

Porous metal is gaining popularity for application as a void filler in bone surgery and a growing number of in vitro- and preclinical in vivo studies are being published. It is understandable that the transition from the long-standing implantation of bioresorbable materials like calcium phosphate to implantation of recently available non resorbable porous metals requires a conservative attitude in the presence of sparse mid-term data and in the absence of a large body of good long-term clinical results. This thesis focuses on the application of particles made of porous, commercially pure (cp) titanium to serve as a full bone substitute material for impaction grafting (Figure 4). This material seems a logical choice based on the above mentioned criteria and specifications: cp titanium as a bulk material is very deformable (ductile) with a strength comparable to stainless steel. Further, cp titanium is biocompatible and osteoconductive. By using a high porosity,

it is possible to create porous titanium scaffolds which are iso-elastic compared to bone. Finally, porous cp titanium can be produced as particles of different sizes in large quantities at an affordable price [81,85-101]. Although the properties of titanium and titanium based alloys are very promising, the only porous metal that has been clinically applied in a large number of orthopaedic patients is porous tantalum (trabecular metal®) [35,37]. Porous tantalum augments are used to fill defects in hip revision surgery with good medium-term (<5 years) results. However, porous tantalum is not available as particles. Besides the report of Allfram et al (cp titanium particles with a diameter of 0.7 – 1.0 mm for application in revision femoral surgery) so far no clinical data have been available about application of porous titanium particles in orthopaedic surgery, and more specifically, in hip replacement surgery [102]. Some clinical data with variable results are available about application of porous titanium particles as void fillers in maxillofacial surgery [103,104].

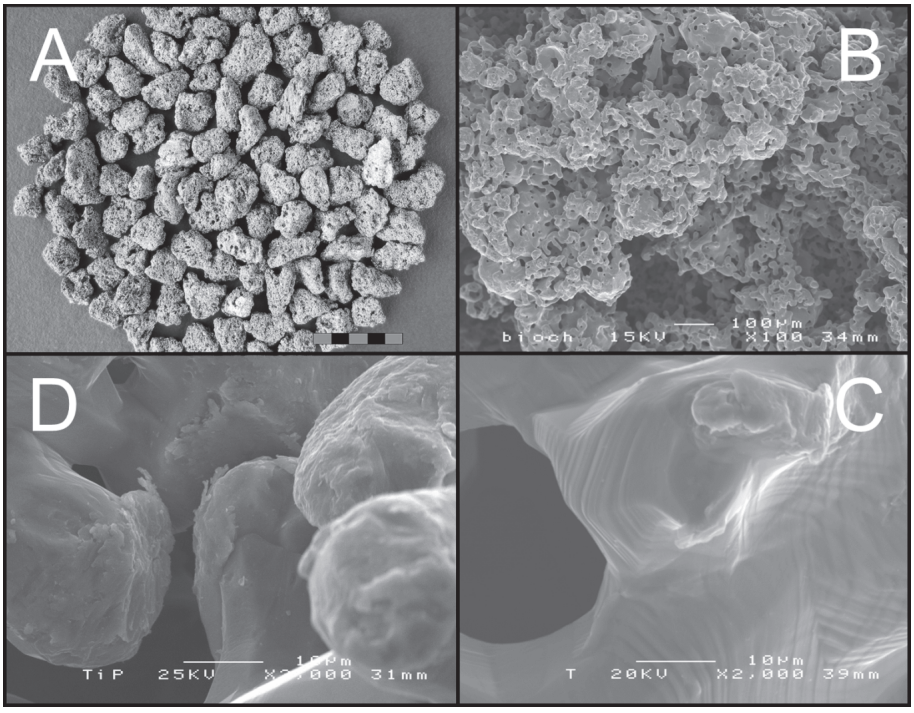


Figure 4 *Porous titanium particles at different magnifications. A: macroscopic view (scale bar indicates 1 cm). B: the irregular particle surface (SEM, scanning electron microscopy, 100x). C and D: crystalline micro surface and flake like micro particles (SEM, 2000x).*

Considerations with respect to porous titanium particles

Impactability and stability

One of the major questions is if porous titanium particles can be impacted like bone graft particles. Although cp titanium is a ductile material, impaction of porous titanium particles might lead to fracturing of the titanium particles themselves or the surrounding bone. Further, gaps might remain between the implanted material and the surrounding bone if the particles do not adapt well to the given defect. Gap size should probably not exceed 2.0 mm [105]. After impaction, impacted particles should not further deform under loaded conditions. Besides resistance to loading, impacted porous titanium particles should also interlock to resist in vivo shear forces. Initial micromotion at the interface between the implanted porous titanium particles and the surrounding bone should be within 40 µm to enable creeping substitution of a flexible interface into a stiff and solid bonding of the implant by osseo-integration with the surrounding bone [106-112]. The question is if the surface geometry of porous titanium particles enables sufficient interlocking with the opposing surface of the osseous defect to minimize micromotion and strains within safe borders for the ingrowth of bone.

Surface characteristics

Adherence of bone precursor cells to the implant surface is a prerequisite for colonization by host tissue and subsequent durable anchoring of the implant. Bony anchoring by osseo-integration seems to be the optimal long-term fixation for mechanically loaded implants [113,114]. From this view, biocompatibility of the implant should include a stimulating environment for attachment, proliferation, differentiation and matrix production by osteoprogenitor cells. These stimulating properties are best for hydroxyapatite (HA), followed by cp Ti and Ti alloys, CoCrMo and ending with resorbable polymers [115]. The excellent biocompatibility of titanium can be further upgraded by roughening of the implant surface [99-101,116,117]. In contrast, the surface of porous titanium particles is smooth which raises the question if porous titanium possess adequate osteoconductive properties.

Pore size

Bone can grow into pores as small as 50 µm, however, the minimal accessible pore size for adequate bone ingrowth seems to be 100 µm [118,119]. Optimal rates for both ingrowth and mechanical fixation have been observed with pore sizes ranging from 100 – 400 µm [118-122]. It is the question if pore sizes within particles (intra-particle pore size) as well as between porous titanium particles (inter-particle pore size) a matrix of impacted porous titanium particles allow for ingrowth of host bone.

Elasticity

Cp titanium is relatively elastic compared to other metals (Table 1). However, solid annealed cp titanium is still six times stiffer than cortical bone and about two hundred to thousand times stiffer than cancellous bone. Although porous titanium particles have a lower stiffness than solid cp titanium, the stiffness of the porous titanium particle matrix is increased by impaction. Deformation due to loading of a fresh implant can stimulate osseo-integration [123]. The question is if a graft layer of impacted porous titanium particles possesses the right elasticity to prevent stress shielding on one hand, and allow enough but not too much deformation during loading to stimulate the ingrowth of host bone.

OUTLINE AND AIMS OF THE THESIS

A series of successive studies was conducted with the aim to evaluate the mechanical and biological suitability of porous titanium particles for reconstruction of defects in hip joint replacement surgery with a cemented impaction grafting technique. The main questions were:

1. Are the handling properties of porous titanium particles sufficient to reconstruct a bone defect in hip joint replacement surgery? CHAPTER TWO
2. Does impaction or loading of porous titanium particles result in micro particle release? CHAPTERS TWO AND THREE
3. Does a reconstructive layer of porous titanium particles provide adequate primary stability to allow full weight bearing without compromising bone ingrowth? CHAPTERS THREE AND FOUR
4. Is a reconstructive layer of impacted TiP penetrated by bone cement during cementation of a total hip prosthesis? CHAPTERS THREE AND FOUR
5. Are porous titanium particles osteoconductive like allograft bone particles? CHAPTER FIVE
6. Does impaction alter the osteoconductivity of porous titanium particles? CHAPTER FIVE
7. Does a surface treatment alter the osteoconductivity of porous titanium particles? CHAPTER FIVE AND SIX
8. Do impacted porous titanium particles result in an osseo-integrated implant? CHAPTER SEVEN
9. Does the implantation of porous titanium particles result in third body wear of the artificial articular surface? CHAPTER SEVEN
10. Does implantation and in vivo loading of porous titanium particles result in systemically elevated Ti concentrations? CHAPTER SEVEN

REFERENCES

1. Grainger R, Cicuttini FM. Medical management of osteoarthritis of the knee and hip joints. Med J Aust 2004;180(5):232-6.
2. Valdes AM, Spector TD. The contribution of genes to osteoarthritis. Med Clin North Am 2009;93(1):45-66, x.
3. Garellick G, Kärrholm J, Rogmark C, Herberts P. Swedish Hip Arthroplasty Register. Annual Report 2010. https://www.shpr.se/en/Publications/DocumentsReports.aspx October 2011.
4. Reginster JY. The prevalence and burden of arthritis. Rheumatology (Oxford) 2002;41 Supp 1:3-6.
5. van der Waal JM, Terwee CB, van der Windt DA, Bouter LM, Dekker J. The impact of non-traumatic hip and knee disorders on health-related quality of life as measured with the SF-36 or SF-12. A systematic review. Qual Life Res 2005;14(4):1141-55.
6. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ 2003;81(9):646-56.
7. Loza E, Lopez-Gomez JM, Abasolo L, Maese J, Carmona L, Batlle-Gualda E, Artrocad Study G. Economic burden of knee and hip osteoarthritis in Spain. Arthritis Rheum 2009;61(2):158-65.
8. March LM, Bachmeier CJ. Economics of osteoarthritis: a global perspective. Baillieres Clin Rheumatol 1997;11(4):817-34.
9. Grotle M, Hagen KB, Natvig B, Dahl FA, Kvien TK. Prevalence and burden of osteoarthritis: results from a population survey in Norway. J Rheumatol 2008;35(4):677-84.
10. Roux CH, Saraux A, Mazieres B, Pouchot J, Morvan J, Fautrel B, Testa J, Fardellone P, Rat AC, Coste J and others. Screening for hip and knee osteoarthritis in the general population: predictive value of a questionnaire and prevalence estimates. Ann Rheum Dis 2008;67(10):1406-11.
11. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M and others. OARSI recommendations for the management of hip and knee osteoarthritis, part I: critical appraisal of existing treatment guidelines and systematic review of current research evidence. Osteoarthritis Cartilage 2007;15(9):981-1000.
12. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M and others. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008;16(2):137-62.
13. Charnley J. The long-term results of low-friction arthroplasty of the hip performed as a primary intervention. J Bone Joint Surg Br 1972;54(1):61-76.
14. Corbett KL, Losina E, Nti AA, Prokopetz JJ, Katz JN. Population-based rates of revision of primary total hip arthroplasty: a systematic review. PLoS One 2010;5(10):e13520.
15. Rasanen P, Paavolainen P, Sintonen H, Koivisto AM, Blom M, Ryynanen OP, Roine RP. Effectiveness of hip or knee replacement surgery in terms of quality-adjusted life years and costs. Acta Orthop 2007;78(1):108-15.
16. Baumann C, Rat AC, Osnowycz G, Mainard D, Cuny C, Guillemin F. Satisfaction with care after total hip or knee replacement predicts self-perceived health status after surgery. BMC Musculoskelet Disord 2009;10:150.

17. Jones CA, Voaklander DC, Johnston DW, Suarez-Almazor ME. Health related quality of life outcomes after total hip and knee arthroplasties in a community based population. J Rheumatol 2000;27(7):1745-52.

18. Mariconda M, Galasso O, Costa GG, Recano P, Cerbasi S. Quality of life and functionality after total hip arthroplasty: a long-term follow-up study. BMC Musculoskelet Disord 2011;12:222.

19. Holzwarth U, Cotogno G. Total Hip Arthroplasty. State of the Art, Challenges and Prospects. http://ihcp.jrc.ec.europa.eu/. 2012.

20. Wainwright C, Theis JC, Garneti N, Melloh M. Age at hip or knee joint replacement surgery predicts likelihood of revision surgery. J Bone Joint Surg Br 2011;93(10):1411-5.

21. Falbrede I, Widmer M, Kurtz S, Schneidmuller D, Dudda M, Roder C. [Utilization rates of lower extremity prostheses in Germany and Switzerland: A comparison of the years 2005-2008]. Orthopade 2011;40(9):793-801.

22. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 2007;89(4):780-5.

23. Otten R, van Roermund PM, Picavet HS. [Trends in the number of knee and hip arthroplasties: considerably more knee and hip prostheses due to osteoarthritis in 2030]. Ned Tijdschr Geneeskd 2010;154:A1534.

24. Jassal SV, Krahn MD, Naglie G, Zaltzman JS, Roscoe JM, Cole EH, Redelmeier DA. Kidney transplantation in the elderly: a decision analysis. J Am Soc Nephrol 2003;14(1):187-96.

25. Lord SJ, Howard K, Allen F, Marinovich L, Burgess DC, King R, Atherton JJ. A systematic review and economic analysis of drug-eluting coronary stents available in Australia. Med J Aust 2005;183(9):464-71.

26. Robertson C, Arcot Ragupathy SK, Boachie C, Dixon JM, Fraser C, Hernandez R, Heys S, Jack W, Kerr GR, Lawrence G and others. The clinical effectiveness and cost-effectiveness of different surveillance mammography regimens after the treatment for primary breast cancer: systematic reviews registry database analyses and economic evaluation. Health Technol Assess 2011;15(34):v-vi, 1-322.

27. 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.

28. de Kam DC, Gardeniers JW, Hendriks JC, Veth RP, Schreurs BW. Cemented polyethylene cups in patients younger than 40 years. Clin Orthop Relat Res 2009;467(7):1753-64.

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

30. Gie GA, Linder L, Ling RS, Simon JP, Slooff TJ, Timperley AJ. Impacted cancellous allografts and cement for revision total hip arthroplasty. J.Bone Joint Surg.Br. 1993;75(1):14-21.

31. Bauer TW. Particles and periimplant bone resorption. Clin Orthop Relat Res 2002(405):138-43.

32. D'Antonio JA, Capello WN, Borden LS, Bargar WL, Bierbaum BF, Boettcher WG, Steinberg ME, Stulberg SD, Wedge JH. Classification and management of acetabular abnormalities in total hip arthroplasty. Clin Orthop Relat Res 1989(243):126-37.

33. Retpen JB, Varmarken JE, Rock ND, Jensen JS. Unsatisfactory results after repeated revision of hip arthroplasty. 61 cases followed for 5 (1-10) years. Acta Orthop Scand 1992;63(2):120-7.

34. Gustke KA. Jumbo cup or high hip center: is bigger better? J Arthroplasty 2004;19(4 Suppl 1):120-3.

35. Ballester Alfaro JJ, Sueiro Fernandez J. Trabecular Metal buttress augment and the Trabecular Metal cup-cage construct in revision hip arthroplasty for severe acetabular bone loss and pelvic discontinuity. Hip Int 2010;20 (Suppl 7)(S7):119-127.

36. Christie MJ, Barrington SA, Brinson MF, Ruhling ME, DeBoer DK. Bridging massive acetabular defects with the triflange cup: 2- to 9-year results. Clin Orthop Relat Res 2001(393):216-27.

37. Flecher X, Sporer S, Paprosky W. Management of severe bone loss in acetabular revision using a trabecular metal shell. J Arthroplasty 2008;23(7):949-55.

38. Garcia-Cimbrelo E, Cruz-Pardos A, Garcia-Rey E, Ortega-Chamarro J. The survival and fate of acetabular reconstruction with impaction grafting for large defects. Clin Orthop Relat Res 2010;468(12):3304-13.

39. Gross AE, Goodman S. The current role of structural grafts and cages in revision arthroplasty of the hip. Clin Orthop Relat Res 2004(429):193-200.

40. Lee JM, Nam HT. 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.

41. Paprosky WG, O'Rourke M, Sporer SM. The treatment of acetabular bone defects with an associated pelvic discontinuity. Clin Orthop Relat Res 2005;441:216-20.

42. Regis D, Magnan B, Sandri A, Bartolozzi P. Long-term results of anti-protrusion cage and massive allografts for the management of periprosthetic acetabular bone loss. J Arthroplasty 2008;23(6):826-32.

43. Shinar AA, Harris WH. Bulk structural autogenous grafts and allografts for reconstruction of the acetabulum in total hip arthroplasty. Sixteen-year-average follow-up. J Bone Joint Surg Am 1997;79(2):159-68.

44. Sporer SM, O'Rourke M, Chong P, Paprosky WG. The use of structural distal femoral allografts for acetabular reconstruction. Average ten-year follow-up. J.Bone Joint Surg.Am. 2005;87(4):760-765.

45. Taunton MJ, Fehring TK, Edwards P, Bernasek T, Holt GE, Christie MJ. Pelvic discontinuity treated with custom triflange component: a reliable option. Clin Orthop Relat Res 2012;470(2):428-34.

46. van Haaren EH, Heyligers IC, Alexander FG, Wuisman PI. High rate of failure of impaction grafting in large acetabular defects. J Bone Joint Surg Br 2007;89(3):296-300.

47. van Egmond N, De Kam DC, Gardeniers JW, Schreurs BW. Revisions of extensive acetabular defects with impaction grafting and a cement cup. Clin Orthop Relat Res 2011;469(2):562-73.

48. 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.

49. 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.

50. Valle CJ, Paprosky WG. Classification and an algorithmic approach to the reconstruction of femoral deficiency in revision total hip arthroplasty. J Bone Joint Surg Am 2003;85-A Suppl 4:1-6.

51. Dohmae Y, Bechtold JE, Sherman RE, Puno RM, Gustilo RB. Reduction in cement-bone interface shear strength between primary and revision arthroplasty. Clin Orthop Relat Res 1988(236):214-20.

52. Gramkow J, Jensen TH, Varmarken JE, Retpen JB. Long-term results after cemented revision of the femoral component in total hip arthroplasty. J Arthroplasty 2001;16(6):777-83.

53. Hultmark P, Karrholm J, Stromberg C, Herberts P, Mose CH, Malchau H. Cemented first-time revisions of the femoral component: prospective 7 to 13 years' follow-up using second-generation and third-generation technique. J Arthroplasty 2000;15(5):551-61.

54. Lie SA, Havelin LI, Furnes ON, Engesaeter LB, Vollset SE. Failure rates for 4762 revision total hip arthroplasties in the Norwegian Arthroplasty Register. J Bone Joint Surg Br 2004;86(4):504-9.

55. Neumann D, Thaler C, Dorn U. Management of Vancouver B2 and B3 femoral periprosthetic fractures using a modular cementless stem without allografting. Int Orthop 2012;36(5):1045-50.

56. Eldridge JD, Smith EJ, Hubble MJ, Whitehouse SL, Learmonth ID. Massive early subsidence following femoral impaction grafting. J Arthroplasty 1997;12(5):535-40.

57. Pekkarinen J, Alho A, Lepisto J, Ylikoski M, Ylinen P, Paavilainen T. Impaction bone grafting in revision hip surgery. A high incidence of complications. J Bone Joint Surg Br 2000;82(1):103-7.

58. Mihalko WM, Beaudoin AJ, Cardea JA, Krause WR. Finite-element modelling of femoral shaft fracture fixation techniques post total hip arthroplasty. J Biomech 1992;25(5):469-76.

59. Holt G, Hook S, Hubble M. Revision total hip arthroplasty: the femoral side using cemented implants. Int Orthop 2011;35(2):267-73.

60. Lamberton TD, Kenny PJ, Whitehouse SL, Timperley AJ, Gie GA. Femoral impaction grafting in revision total hip arthroplasty: a follow-up of 540 hips. J Arthroplasty 2011;26(8):1154-60.

61. Ornstein E, Linder L, Ranstam J, Lewold S, Eisler T, Torper M. Femoral impaction bone grafting with the Exeter stem - the Swedish experience: survivorship analysis of 1305 revisions performed between 1989 and 2002. J Bone Joint Surg Br 2009;91(4):441-6.

62. Pak JH, Paprosky WG, Jablonsky WS, Lawrence JM. Femoral strut allografts in cementless revision total hip arthroplasty. Clin Orthop Relat Res 1993(295):172-8.

63. Schreurs BW, Arts JJ, Verdonschot N, Buma P, Slooff TJ, Gardeniers JW. Femoral component revision with use of impaction bone-grafting and a cemented polished stem. Surgical technique. J Bone Joint Surg Am 2006;88 Suppl 1 Pt 2:259-74.

64. Sierra RJ, Charity J, Tsiridis E, Timperley JA, Gie GA. The use of long cemented stems for femoral impaction grafting in revision total hip arthroplasty. J Bone Joint Surg Am 2008;90(6):1330-6.

65. Vastel L, Lemoine CT, Kerboull M, Courpied JP. Structural allograft and cemented long-stem prosthesis for complex revision hip arthroplasty: use of a trochanteric claw plate improves final hip function. Int Orthop 2007;31(6):851-7.

66. Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg Am 2002;84-A(5):716-20.

67. Conrad EU, Gretch DR, Obermeyer KR, Moogk MS, Sayers M, Wilson JJ, Strong DM. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg Am 1995;77(2):214-24.

68. Cook SD, Salkeld SL, Prewett AB. Simian immunodeficiency virus (human HIV-II) transmission in allograft bone procedures. Spine (Phila Pa 1976) 1995;20(12):1338-42.

69. Galea G, Kopman D, Graham BJ. Supply and demand of bone allograft for revision hip surgery in Scotland. J Bone Joint Surg Br 1998;80(4):595-9.

70. Hooten JP, Jr., Engh CA, Heekin RD, Vinh TN. Structural bulk allografts in acetabular reconstruction. Analysis of two grafts retrieved at post-mortem. J Bone Joint Surg Br 1996;78(2):270-5.

71. Linder L. Cancellous impaction grafting in the human femur: histological and radiographic observations in 6 autopsy femurs and 8 biopsies. Acta Orthop Scand 2000;71(6):543-52.

72. Ullmark G, Obrant KJ. Histology of impacted bone-graft incorporation. J Arthroplasty 2002;17(2):150-7.

73. van der Donk S, Buma P, Slooff TJ, Gardeniers JW, Schreurs BW. Incorporation of morselized bone grafts: a study of 24 acetabular biopsy specimens. Clin.Orthop.Relat Res. 2002(396):131-141.

74. van der Donk S, Weernink T, Buma P, Aspenberg P, Slooff TJ, Schreurs BW. Rinsing morselized allografts improves bone and tissue ingrowth. Clin Orthop Relat Res 2003(408):302-10.

75. Wang JS, Tagil M, Aspenberg P. Load-bearing increases new bone formation in impacted and morselized allografts. Clin Orthop Relat Res 2000(378):274-81.

76. Weiland AJ, Phillips TW, Randolph MA. Bone grafts: a radiologic, histologic, and biomechanical model comparing autografts, allografts, and free vascularized bone grafts. Plast Reconstr Surg 1984;74(3):368-79.

77. Ullmark G, Sorensen J, Langstrom B, Nilsson O. Bone regeneration 6 years after impaction bone grafting: a PET analysis. Acta Orthop 2007;78(2):201-5.

78. Timoshenko S, Goodier J. Theory of Elasticity. Second edition. New York: McGraw-Hill Book company; 1951.

79. Gonzalez-Lomas G, Cassilly RT, Remotti F, Levine WN. Is the etiology of pretibial cyst formation after absorbable interference screw use related to a foreign body reaction? Clin Orthop Relat Res 2011;469(4):1082-8.

80. Landes CA, Ballon A, Roth C. Maxillary and mandibular osteosyntheses with PLGA and P(L/DL)LA implants: a 5-year inpatient biocompatibility and degradation experience. Plast Reconstr Surg 2006;117(7):2347-60.

81. Arts JJ, Gardeniers JW, Welten ML, Verdonschot N, Schreurs BW, Buma P. No negative effects of bone impaction grafting with bone and ceramic mixtures. Clin.Orthop.Relat Res. 2005;438:239-247.

82. Arts JJ, Schreurs BW, 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. Mech.Eng [H.] 2005;219(4):257-263.

83. Blom AW, Wylde V, Livesey C, Whitehouse MR, Eastaugh-Waring S, Bannister GC, Learmonth ID. Impaction bone grafting of the acetabulum at hip revision using a mix of bone chips and a biphasic porous ceramic bone graft substitute. Acta Orthop 2009;80(2):150-4.

84. Bolder SB, Verdonschot N, Schreurs BW, 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.

85. van Haaren EH, Smit TH, Phipps K, Wuisman PI, Blunn G, Heyligers IC. 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(2):267-71.

86. Broz ME, VanderHart DL, Washburn NR. Structure and mechanical properties of poly(D,L-lactic acid)/poly(epsilon -caprolactone) blends. Biomaterials 2003;24(23):4181-90.

87. Buckwalter JA, Einhorn TA, Simon SR. Orthopaedic Basic Science: AAOS; 2000.

88. Disegi JA, Eschbach L. Stainless steel in bone surgery. Injury 2000;31 Suppl 4:2-6.

89. Engelberg I, Kohn J. Physico-mechanical properties of degradable polymers used in medical applications: a comparative study. Biomaterials 1991;12(3):292-304.

90. Eschbach L. Nonresorbable polymers in bone surgery. Injury 2000;31 Suppl 4:22-7.

91. Eshraghi S, Das S. Mechanical and microstructural properties of polycaprolactone scaffolds with one-dimensional, two-dimensional, and three-dimensional orthogonally oriented porous architectures produced by selective laser sintering. Acta Biomater 2010;6(7):2467-76.

92. Frosch KH, Sturmer KM. Metallic Biomaterials in Skeletal Repair. Eur J Trauma 2006;32:149-59.

93. Hvid I. Trabecular bone strength at the knee. Clin Orthop Relat Res 1988;227:210-21.

94. Ioku K, Yoshimura M, Somiya S. Microstructure and mechanical properties of hydroxyapatite ceramics with zirconia dispersion prepared by post-sintering. Biomaterials 1990;11(1):57-61.

95. Knepper M, Moricca S, Milthorpe BK. Stability of hydroxyapatite while processing short-fibre reinforced hydroxyapatite ceramics. Biomaterials 1997;18(23):1523-9.

96. Li JP, Li SH, Van Blitterswijk CA, de Groot K. Cancellous bone from porous Ti6Al4V by multiple coating technique. J Mater Sci Mater Med 2006;17(2):179-85.

97. Ling JP. Porous Titanium for Biomedical Applications. Thesis. WPS, Zutphen, The Netherlands; 2004.

98. Long M, Rack HJ. Titanium alloys in total joint replacement--a materials science perspective. Biomaterials 1998;19(18):1621-39.

99. Pohler OE. Unalloyed titanium for implants in bone surgery. Injury 2000;31 Suppl 4:7-13.

100. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. Biomaterials 2005;26(30):6014-23.

101. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Matsushita T, Kokubo T, Nakamura T. Osteoinductive porous titanium implants: effect of sodium removal by dilute HCl treatment. Biomaterials 2006;27(13):2682-91.

102. Alffram PA, Bruce L, Bjursten LM, Urban RM, Andersson GB. 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.

103. Holmberg L, Forsgren L, Kristerson L. Porous titanium granules for implant stability and bone regeneration - a case followed for 12 years. Ups J Med Sci 2008;113(2):217-20.

104. Lambert F, Lecloux G, Leonard A, Sourice S, Layrolle P, Rompen E. Bone Regeneration Using Porous Titanium Particles versus Bovine Hydroxyapatite: A Sinus Lift Study in Rabbits. Clin Implant Dent Relat Res 2011.

105. Dalton JE, Cook SD, Thomas KA, Kay JF. The effect of operative fit and hydroxyapatite coating on the mechanical and biological response to porous implants. J Bone Joint Surg Am 1995;77(1):97-110.

106. Cullinane DM, Fredrick A, Eisenberg SR, Pacicca D, Elman MV, Lee C, Salisbury K, Gerstenfeld LC, Einhorn TA. Induction of a neoarthrosis by precisely controlled motion in an experimental mid-femoral defect. J Orthop Res 2002;20(3):579-86.

107. Isaksson H, Wilson W, van Donkelaar CC, Huiskes R, Ito K. Comparison of biophysical stimuli for mechano-regulation of tissue differentiation during fracture healing. J Biomech 2006;39(8):1507-16.

108. Jasty M, Krushell R, Zalenski E, O'Connor D, Sedlacek R, Harris W. The contribution of the nonporous distal stem to the stability of proximally porous-coated canine femoral components. J Arthroplasty 1993;8(1):33-41.

109. Kapur S, Baylink DJ, Lau KH. Fluid flow shear stress stimulates human osteoblast proliferation and differentiation through multiple interacting and competing signal transduction pathways. Bone 2003;32(3):241-51.

110. Nagel T, Kelly DJ. Mechano-regulation of mesenchymal stem cell differentiation and collagen organisation during skeletal tissue repair. Biomech Model Mechanobiol 2010;9(3):359-72.

111. Pilliar RM, Lee JM, Maniatopoulos C. Observations on the effect of movement on bone ingrowth into porous-surfaced implants. Clin Orthop Relat Res 1986(208):108-13.

112. Yan YX, Gong YW, Guo Y, Lv Q, Guo C, Zhuang Y, Zhang Y, Li R, Zhang XZ. Mechanical strain regulates osteoblast proliferation through integrin-mediated ERK activation. PLoS One 2012;7(4):e35709.

113. De Man FH, Tigchelaar W, Marti RK, Van Noorden CJ, Van der Vis HM. 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.

114. Viceconti M, Monti L, Muccini R, Bernakiewicz M, Toni A. Even a thin layer of soft tissue may compromise the primary stability of cementless hip stems. Clin Biomech (Bristol, Avon) 2001;16(9):765-75.

115. Endres S, Landgraff M, Kratz M, Wilke A. [Biocompatibility testing of various biomaterials as dependent on immune status]. Z Orthop Ihre Grenzgeb 2004;142(3):358-65.

116. Ungersbock A, Pohler O, Perren SM. Evaluation of the soft tissue interface at titanium implants with different surface treatments: experimental study on rabbits. Biomed Mater Eng 1994;4(4):317-25.

117. Ungersbock A, Pohler OE, Perren SM. Evaluation of soft tissue reactions at the interface of titanium limited contact dynamic compression plate implants with different surface treatments: an experimental sheep study. Biomaterials 1996;17(8):797-806.

118. Itala AI, Ylanen HO, Ekholm C, Karlsson KH, Aro HT. Pore diameter of more than 100 microm is not requisite for bone ingrowth in rabbits. J Biomed Mater Res 2001;58(6):679-83.

119. Jones AC, Arns CH, Hutmacher DW, Milthorpe BK, Sheppard AP, Knackstedt MA. The correlation of pore morphology, interconnectivity and physical properties of 3D ceramic scaffolds with bone ingrowth. Biomaterials 2009;30(7):1440-51.

120. Bobyn JD, Pilliar RM, Cameron HU, Weatherly GC. The optimum pore size for the fixation of porous-surfaced metal implants by the ingrowth of bone. Clin Orthop Relat Res 1980(150):263-70.

121. Chang BS, Lee CK, Hong KS, Youn HJ, Ryu HS, Chung SS, Park KW. Osteoconduction at porous hydroxyapatite with various pore configurations. Biomaterials 2000;21(12):1291-8.

122. Welsh RP, Pilliar RM, Macnab I. Surgical implants. The role of surface porosity in fixation to bone and acrylic. J Bone Joint Surg Am 1971;53(5):963-77.

123. Leucht P, Kim JB, Wazen R, Currey JA, Nanci A, Brunski JB, Helms JA. Effect of mechanical stimuli on skeletal regeneration around implants. Bone 2007;40(4):919-30.

CHAPTER TWO

IMPACTABILITY AND TIME DEPENDENT MECHANICAL PROPERTIES OF POROUS TITANIUM PARTICLES FOR APPLICATION IN IMPACTION GRAFTING

J Biomed Mater Res B Appl Biomater 2010;95(1):131-40.

ABSTRACT

Aims: Impaction grafting with bone particles is a successful technique to restore bone stock loss during hip revision surgery. Allograft shortage is expected within the near future. This study investigates the feasability of porous titanium particles (TiP) to replace bone particles (BoP) and to compare mechanical properties of TiP and a commercially available porous ceramic bone graft extender (CeP). Impactability and time-dependent mechanical properties (stability and stiffness during physiologic loading (0.1 – 2.5 MPa)) were assessed by standardized impaction and a confined compression test. Loaded samples were used for particle release analysis.

Findings: TiP were more impactable than BoP and created a stable, highly entangled macro porous construct. CeP were crushed during impaction, resulting in non-cohesive specimens of small ceramic particles. TiP showed very little deformation at the end of physiological loading. Impacted TiP were stiffer than BoP but more elastic than CeP. TiP generated a low volume of micro particles (0.2% of original TiP weight) with a bimodal size distribution (diameter range 7–2000 μm).

Conclusion: TiP are impactable and create a stable, elastic and highly entangled, macro porous layer. Further in vitro testing and biological studies are warranted to verify whether the promising results are maintained with THA reconstructions.

INTRODUCTION

Total hip arthroplasty (THA) is a very successful orthopedic intervention. The prevalence of those aged over 55 with existing hip replacements is estimated at 32/1000 [1]. As patients become more demanding and survival characteristics of available hip prostheses are favourable and more predictable nowadays, the age at first implantation declines. Because of the implantation in younger and more active patients, the implants are subjected to heavier loading during an increasing number of years, resulting in a higher incidence of failures of THA [2]. The revision burden as defined by the number of revisions divided by the total number of primary and revision operations, amounted in the US to nineteen percent from 1997 – 2003 [3].

During revision surgery one of the major encountered problems is profound osteolysis and bone stock restoration is one of the key factors for long-term stability of revised implants. A successful technique to restore local bone stock is bone impaction grafting (BIG) in which the original bone stock is restored by impaction of autograft or allograft bone chips in the bony defects. In cases of larger defects a metal mesh can be used to ensure containment of grafts. BIG has shown promising long-term results on both the acetabular and femoral side with a survival rate comparable to primary reconstructions [4,5].

Usually only human bone chips are used for BIG. There is a number of reasons to search for an alternative bone graft material. First, allograft shortage is expected in the near future as a result of the increasing incidence of primary THA's and the strongly increasing number of revision arthroplasties [6]. Second, the application of allografts and xenografts carries the potential hazard of disease transmission and can have religious objections [7,8]. Finally, it may take several years before bone grafts are gradually replaced by host bone at the interface between graft layer and cement where stressess are high [9-12]. A synthetic bone substitute that can be produced in large quantities could solve these limitations.

Resorbable calcium phosphate compounds, which are available in different biphasic compositions and porosities, have been applied in a wide range of orthopaedic interventions [13-16]. Although Oonishi has successfully applied hydroxy-apatite particles as a 100 percent bone graft substitute in hip revision surgery, the application of bioceramic particles in BIG has some drawbacks [16-21]. First, handling is quite

difficult as bioceramic particles are hard, brittle and do not stick together very well during impaction. Second, an increased peri-operative fracture risk as a consequence of peak stresses in the surrounding bone during impaction has been reported [18-20]. Furthermore, the application of pure bioceramic particles in BIG results in particle pulverization and excessive cement penetration [18,19]. For these reasons Arts et al advocate the application of biphasic calcium phosphate as a graft extender in BIG instead of using it as a full bone graft substitute [22,23]. However, Arts et al found in a pre-clinical study with a biphasic calciumphosphate bone graft extender that many pulverized particles were found in a fibrous tissue interface between the graft layer and the cemented prosthesis and that these particles were engulfed by multinucleated cells as early as 15 weeks postoperatively [23]. From this perspective the application of a bioceramic bone graft extender in bone impaction grafting could hamper the mechanical integrity of a reconstruction by degradation of the carrying material and by deposition of a biomechanically inferior thick fibrous tissue layer between cemented components and the reconstructive layer within months after surgery [23,24].

A suitable biomaterial for impaction grafting should be deformable and ductile in order to 'shape' the construct and to minimize the intra-operative fracture risk as seen with brittle and hard bioceramics. After impaction the graft material should show limited further plastic deformation under in vivo loading conditions. From a biological perspective, the material should be porous and elastic to promote armouring of the graft layer by invading fibrous tissue and bone tissue and to prevent the formation of peak stressess at the interface of the reconstruction with the surrounding bone. A non-degradable material should not be a disadvantage persé as quick resorption of a bone substitute material could interfere with osteoconduction and reconstruction integrity

Highly porous titanium particles (TiP) may be a potential non resorbable bone graft substitute for impaction grafting fulfilling the requirements as described above. The ductile behavior of titanium combined with a high porosity results in a highly deformable material. TiP can be made in a wide range of sizes, which enables application in all kinds of defects encountered during revision surgery. Furthermore, titanium is known as a biocompatible material, it can be coated to optimize osteoconduction, and is used for the manufacturing of orthopaedic and dental implantants as well as ostheosynthetic devices like plates and screws [25-28]. The osteoconductive properties of titanium enable direct

implant-to-bone contact and the fixation strength to the surrounding bone is superior to stainless steel implants [29,30]. Therefore, TiP could be a potential material to serve as a bone substitute in impaction grafting from both a mechanical and biological perspective. In this study, we assessed the feasibility of using porous titanium particles for impaction grafting. More specifically we addressed the following four questions:

1. Are porous titanium particles impactable like allografts and how is handling under conditions simular to BIG?
2. How much do porous titanium particles deform during physiological loading after impaction (visco-elastic behavior) compared to allografts and porous calcium phosphate ceramic particels?
3. How stiff is a graft layer of impacted porous titanium particles compared to impacted allografts and impacted porous calcium phosphate ceramic particles?
4. How much debris is generated during impaction and what is the size of these debris particles?

To answer these four questions we subjected porous titanium particles, porous calcium phosphate ceramic particles and allografts to standardized impaction and a subsequent loading regime in a previously described in vitro model [13]. In addition, particulate debris was quantified and analyzed with respect to particle size distribution.

MATERIALS AND METHODS

Materials

Three materials were used: porous titanium particles (TiP, Hereford Metal Powder Company Ltd, Hereford, UK), ceramic particles (CeP, BoneSave®, Stryker Howmedica Osteonics, Limerick, Ireland) and morsellized human cancellous bone particles (BoP) (Table 1, Figure 1).

The porous titanium particles as used in this study were produced during the purification of titanium through titanium tetrachloride ($TiCl_4$). This process creates porous commercially pure titanium (cpTi) with a crystalline microtexture and interconnected pores (Figure 1). TiP have heterogeneous shapes ranging from round to irregular (Figure

1). BoneSave® is a commercially available bioceramic that is constituted of 80% β–TCP and 20% HA and with a non interconnected porosity of 50% [31]. Morsellized cancellous bone chips (BoP) were obtained by nibbling the cancellous bone of five freshly frozen (-80°C) human femoral heads with a rongeur. CeP have a surface porosity of 50%. From cross-sectional photographs (SEM, Jeol 6310 scanning electron microscope) the surface porosity of individual TiP and BoP used in this study was calculated to be 83 ± 2% (mean ± SD) and 75 ± 4% respectively.

graft	material	diameter (mm)	porosity (%)	inter-connective
TiP	commercially pure titanium	2 – 4	83 %	yes
CeP	TCP/HA 80/20	1.4 – 2.8	50 %	no
BoP	cancellous human bone	4 – 6	75 %	yes

Table 1 *Graft characteristics: materials, corresponding particle size, measured particle cross-sectional surface porosity (SEM).*

Methods

Particle size distribution before impaction

20 grams of TiP (dry material), 20 grams of CeP (dry material) and 8.2 grams of defatted BoP (corresponding to 20 grams of non-defatted BoP) were sieved with a Retsch VS1000 apparatus (sieve pore diameter in mm: 0; 0.4; 0.7; 1.4; 2.0; 2.8; 4.0; 4.5; 5.0; 5.6; 6.6; 8.0) and corresponding weights were plotted in a graph (Figure 2). Sieved materials were not re-used for other parts of this study.

Impaction

TiP were rinsed (Procedure Number PS03-016; CAM Implants BV, Leiden, The Netherlands) and dried (at 50 ^{0} C during 24 hours) before use. The test chamber and impactors were also rinsed (same procedure). CeP were used as delivered in a sterile batch. BoP used for mechanically tested specimens were not defatted and impacted after adapting to a temperature of 30 ^{0} C. BoP used for analysis of particle generation were defatted before impaction by immersion in acetone at 37 ^{0} C during 24 hours and subsequently dried at 37 ^{0} C during 24 hours. To simulate in vivo handling characteristics, all material were were soaked during thirty minutes before impaction. For practical reasons demineralized water was used. All tests were performed at room temperature. In order to obtain comparable

graft layer thickness, different amounts of dry materials were used depending on the tested graft group: TiP 3000 mg, CeP 4000 milligrams, BoP 1.7 grams (corresponding to 4.0 grams fresh BoP). Tested particle sizes differed per material. This was done for several reasons with respect to realistic application and size of the testing chamber. By in vitro testing we selected the TiP particle size that handled best for application in human reconstructions. Sieving showed that these TiP ranged from 2 – 4 mm (the particles passed a 2 mm pore sieve but were stopped by a 4 mm pore sieve). Arts et al used CeP labeled ''4 – 8 mm'' for realistic in vitro acetabular reconstructions [19]. However, the test chamber did not allow for homogeneous distribution of these large sized non-deformable particles. Therefore, smaller CeP labeled ''2 – 4 mm'' were used. (Figure 1). Sieving of CeP showed an actual diameter range of 1.4 – 2.8 mm, corresponding to the smaller size compared to TiP. BoP were large as recommended for clinical application by Bolder et al and Dunlop et al [32,33] (Figure 1). Despite the larger size of 4.0 – 5.6 mm, homogeneous distribution was possible by manual positioning in the test chamber.

Particles were impacted in a cylindrical brass chamber with a diameter of 20.5 mm (Figure 3). A specially designed impactor was used for standardized impaction of the grafts. The diameter of the impactor was only slightly smaller than the diameter of the test chamber. To allow for free removal of fat and fluid out of the grafts and the test chamber during impaction, three release channels with a diameter of 2.0 mm were made to the side of the impactor. All specimens were impacted by dropping a weight of 420 grams thirty times from a height of 35 centimeters. This results in a degree of impaction of bonegrafts similar to impaction in a sawbone acetabulum by an experienced orthopaedic surgeon (BWS). Height before impaction and after impaction was measured with a marking gauge (resolution 0.05 mm). To determine the impactability of the materials the impaction strain was calculated. Height of the specimen before impaction was indicated by 'h_{init}' , height directly after impaction and at the beginning of loading was indicated by 'h_0' . The impaction strain was calculated as:

$$\varepsilon_{impaction} = [\ln (h_{init} / h_0)]$$

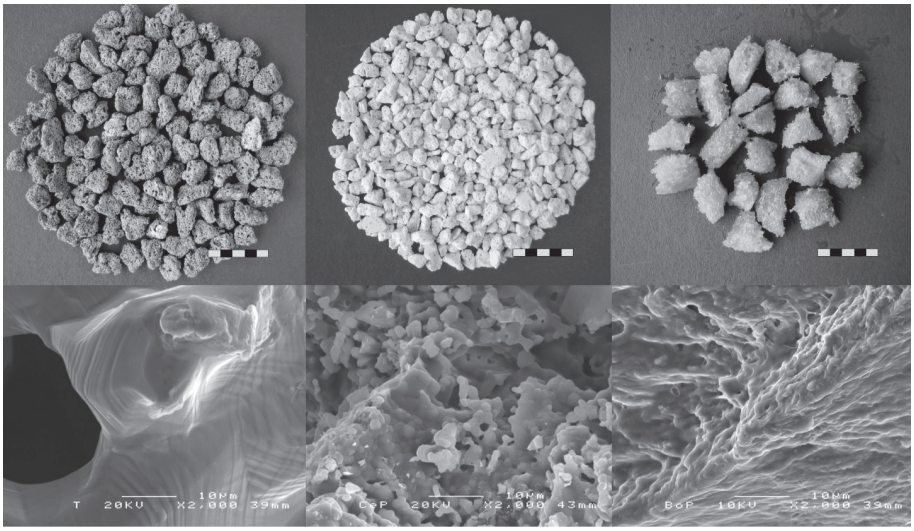


Figure 1 *Top left to right: TiP (3.0 g), CeP (4.0 g) and BoP (4.0 g), before impaction. The bar indicates 1 centimeter. Bottom left to right: close-up of the corresponding material surface (SEM, 2000x). See page 195 for color figure.*

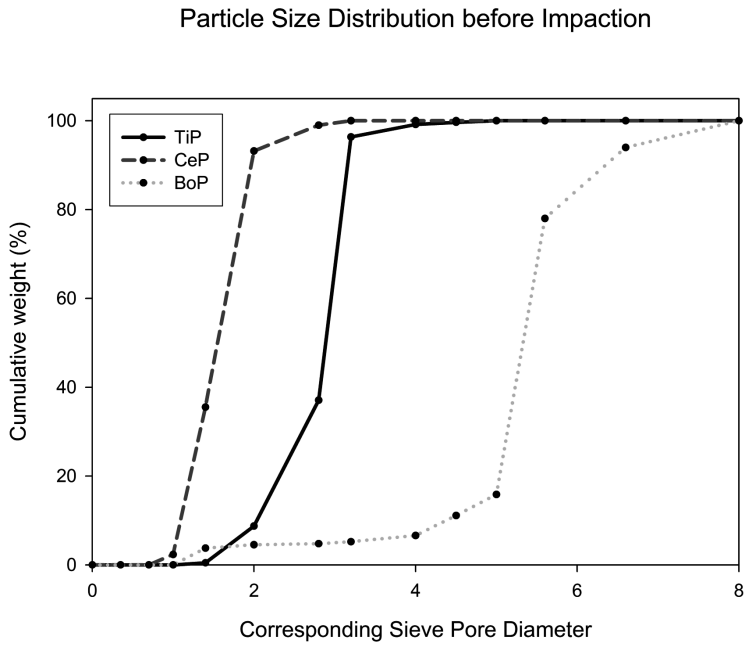


Figure 2 *Particle size distribution before impaction.*

Loading

Directly after impaction the resulting graft layer was loaded in the same chamber with a confined compression test (CCT). The CCT was used previously and measures the deformation and stiffness of the graft layer during loading, and the visco-elastic recoil of the graft layer during subsequent unloading (relaxation) [13]. After impaction, a frame was placed on top of the test chamber with a rigid porous filter on top of the specimen to allow free fluid exudation during loading. On top of the filter a load spreader was placed to ensure that the applied load was equally distributed over the whole surface of the specimen. The specimen was subjected to cyclic loading (0.1 – 2.5 MPa (20 – 840 N)), at a frequency of 1 Hz during 900 seconds while measuring deformation and stiffness of the graft specimen. The applied loading corresponds with stress levels that may be expected around cemented implants and was applied by a servo-hydraulic MTS machine (MTS® Systems Corporation, Minnesota, US) [34,35]. An extensometer, connected between the loading rod and the specimen, measured the height of the specimen during the test. A load cell was placed under the chamber to register the applied load (Figure 3).

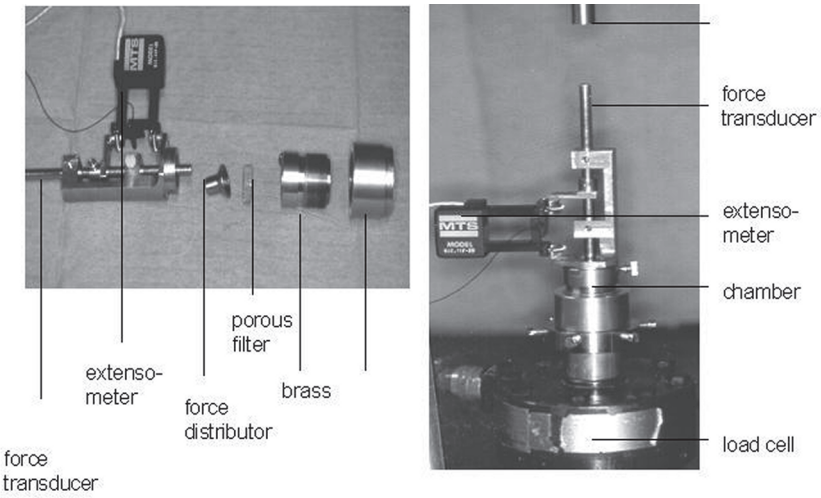


Figure 3 *The testing chamber and the force transducer with the extensometer before and after assembly.*

The loading strain represents the deformation of the materials under dynamic loading. Bone grafts show creep behavior: the specimen height diminishes during the loading period. During every loading cycle the height of the specimens was recorded at minimum stress ($h_{minimum\ stress}$, height at 0.1 MPa) and at maximum stress ($h_{maximum\ stress}$, height at 2.5 MPa). The loading strain was calculated as:

$$\varepsilon_{loading} = [\ln (h_{minimal\ stress} / h_0)]$$

The loading strain was determined for every loading cycle and statistically compared for the values obtained at the end of the loading phase. After 900 seconds of loading the specimen was allowed to relaxate (0 N load) during 900 seconds to measure the visco-elastic recoil.

The cyclic elastic modulus represents the stiffness of a material. The elastic modulus is calculated from the change in stress within one loading cycle (cyclic stress) and the corresponding resulting deformation within the same loading cycle (cyclic strain). The cyclic stress, σ_{cyclic}, was calculated as the difference between minimum stress (0.1 MPa) and maximum stress (2.5 MPa) and remained constant for every loading cycle (2.4 MPa). The cyclic strain, ε_{cyclic}, was calculated as:

$$\varepsilon_{cyclic} = [h_{minimal\ stress} - h_{maximum\ stress}] / [h_{minimal\ stress}]$$

The cyclic elastic modulus, E, was calculated as the ratio of the cyclic stress and cyclic strain:

$$E = [\sigma_{cyclic} / \varepsilon_{cyclic}]$$

Because the cyclic stress was constant, the stiffness was inversely proportional to the cyclic deformation. The stiffness was determined during the whole loading period and statistically compared for the values obtained at the end of the loading phase. Mechanically tested specimens were not re-used for other parts of this study. To judge the interparticle entanglement which is requirement for an adequate reconstructive behavior, some extra specimens were impacted. These specimens were not used for mechanical testing and were removed from the test chamber for macroscopical evaluation directly after impaction (Figure 4).

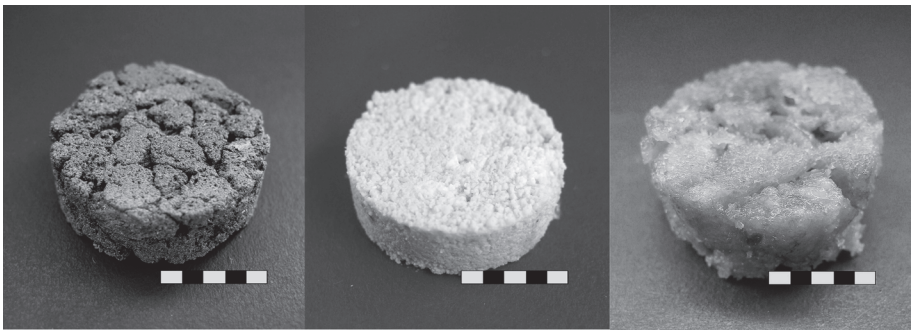


Figure 4 *Impacted specimens of TiP (firmly entangled), CeP (no entanglement) and BoP (some entanglement). The bar represents 1 centimeter. See page 195 for color figure.*

Impaction debris and particle analysis: preparation of specimens.

Specimens were made from 3.0 grams of TiP (rinsed and dried), 4.0 grams of CeP (as originally delivered) and 1.7 grams of BoP (defatted and dried, corresponding to 4.0 grams of fresh BoP). Materials were soaked in demineralized water during thirty minutes at room temperature before impaction. Six specimens were created for every group (n = 6) by impaction as described before.

Impaction debris: particle collection and analysis.

After impaction, specimens were removed from the confined compression chamber. Four specimens of each material were used for quantification of impaction debris weight and particle size analysis. These specimens were ultrasonically rinsed (Bransonic B2210E-MT, Branson Ultrasonic Corporation, US) during fifteen minutes in sterile polypropylene tubes (Ø 30mm, Greiner Bio-One BV, Netherlands) filled with 12,5 ml of demineralized water to remove particles generated during the impaction procedure. CeP specimens desintegrated into small particles during submersion in demineralized water. TiP and BoP specimens were removed from the polypropylene tubes after rinsing and dried (at 50 ^{0} C and 37 ^{0} C respectively during 24 hours). Dry weight loss was measured to quantify the weight of the generated impaction debris of TiP and BoP. The debris in the twelve polypropylene tubes was analyzed with a laser diffraction particle sizer (Malvern Mastersizer® 2000, Malvern Instruments Ltd, Worcestershire, UK). Two specimens of each material were not rinsed after impaction and used for physicochemical analysis

of cross sectioned specimens (embedded in polymethylmethacrylate) and generated particles with a scanning electron microscope (SEM, Jeol 6310, Jeol, Tokyo, Japan) which was equipped with an energy disperse X-ray detector (EDS).

Statistical analysis

Power analysis showed that group size could be as small as four in order to detect a difference in loading strain of 0.05 or a difference in elasticity of 100 MPa ($\alpha = 0.05$ with a power of $\beta = 0.8$) [13]. A group size of five ($n = 5$) was chosen to show relevant differences in loading strain and stiffness between the three used material groups. All measurement calculations for mechanical parameters were expressed as mean ± one standard deviation. Statistical analysis was performed with SPSS 9.0. Differences between groups were analyzed by analysis of variance (ANOVA) and post hoc t-tests (Tukey HSD).

RESULTS

Particle size distribution before impaction

All materials showed a unimodal size distribution. BoP diameter was about 2 – 2.5 times larger than TiP and CeP diameter (Figure 2).

Impaction

Soaked TiP and CeP were poured in the test chamber. The water film made the unimpacted TiP and CeP stick together quite well. Height before impaction was measured. During impaction, water was expelled from TiP through the side channels of the impactor without visible micro particles. The water that was expelled during impaction of CeP had a milky aspect and contained a lot of minute ceramic particles. BoP released a lot of fat during impaction. TiP and CeP were more impactable than BoP (Table 2,3). After impaction, TiP formed a unified cylinder which maintained nicely its shape. The firm entanglement of impacted TiP created homogeneous macro porous ‘cookies’ which were very cohesive and could not be broken easily (Figure 4). Impacted specimens of BoP were less cohesive than specimens of TiP but stuck together after removal from the test chamber. Impacted CeP specimens tended to desintegrate and fell apart quite easily.

graft	height before impaction	height after impaction	impaction strain
TiP	16.5 (0.3)	7.5 (0.2)	0.78 (0.03)
CeP	14.7 (0.2)	6.9 (0.1)	0.76 (0.02)
BoP	15.7 (0.6)	9.8 (0.4)	0.47 (0.01)

Table 2 *Specimen height in millimeters and impaction strain.*

graft	loading strain	relaxation strain	stiffness (MPa)
TiP	0.009 (0.001)	0.009 (0.001)	209 (20)
CeP	0.017 (0.002)	0.009 (0.002)	334 (47)
BoP	0.29 (0.05)	0.10 (0.02)	80 (18)

Table 3 *Loading strain, stiffness and relaxation strain.*

Loading

TiP showed almost no deformation during physiological loading (loading strain 0.009 ± 0.001) (Table 3). CeP deformed twice as much as TiP (loading strain 0.017 ± 0.002, $p < 0.001$) which was still considerably smaller than deformation of BoP (loading strain 0.29 ± 0.05).

After a setting phase of about fifty loading cycles, the stiffness remained almost unchanged during the rest of the loading period for all groups. There was a clear and significant difference between the three tested materials (Table 3). TiP showed an intermediate stiffness (209 ± 20 MPa) and were about 2.5 times as stiff as BoP (80 ± 18 MPa, $p < 0.001$). CeP were about 4 times as stiff as BoP (334 ± 47 MPa, $p < 0.001$) and therefore stiffer than TiP ($p < 0.001$). After unloading the TiP showed no residual deformation: the strain after stress relaxation was equal to the loading strain (0.009 ± 0.001). About 50% of the height loss of CeP during loading recovered during the unloading phase. Although BoP specimens showed a large relaxation strain of 0.10 ± 0.02, this was still only one third of the loading strain (0.29 ± 0.05). During relaxation the bone grafts partially resorbed the fat that was forced out during cyclic loading. The visco-elastic behavior of BoP is shown in Figure 5.

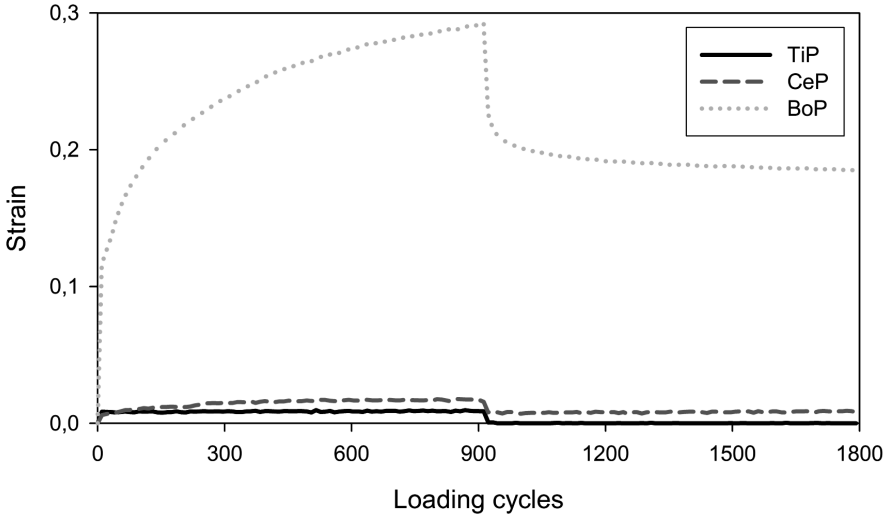


Figure 5 *Mean strain curves during loading and relaxation impacted specimens.*

Impaction debris: particle collection and analysis.

Weight analysis of impacted and dried TiP and BoP specimens showed that the weight loss after impaction was minimal for TiP (0.19 ± 0.09%) and small for BoP (2.7 ± 0.8%). CeP specimens consisted completely of desintegrating small particles and were classified as 100% impaction debris. Laser diffraction of debris specimens showed a bimodal particle size distribution for CeP (peak volumes at 0.5 μm and 500 μm) and BoP (peak volumes at 1 μm and 200 μm) and a quadrimodal particle size distribution for TiP (peak volumes at 13 μm, 250 μm, 700 μm and 1500 μm) (Figure 6). SEM of impacted TiP specimens before imbedding in PMMA revealed a potential source of debris consisting of small titanium flakes (Ø 1-5 μm) still attached to the surface of particles at the site of collision (magnification 2000x, Figure 7). No TiP micro particles were visualized in cross sections and no fracturing of particles was observed even after considerable deformation (magnification 25x). Deformation of TiP was mainly seen at the periphery of the particles with interdigitation of adjacent surfaces, consistent with the good entanglement of impacted specimens. Cross-sections showed a macro porous layer with many inter-particle pores, no fracturing of particles and no impaction debris. BoP specimens were macro porous as well and showed many fragmented parts. Cross-sections of CeP showed that the original particles

had been pulverized during impaction, resulting in a microporous layer of small particles. EDS showed that virtually all debris particles consisted of tested materials (Ti, Ca, P). Very few particles originated from the test chamber (Cu-Zn) or the impactor (Fe-Cr-Ni).

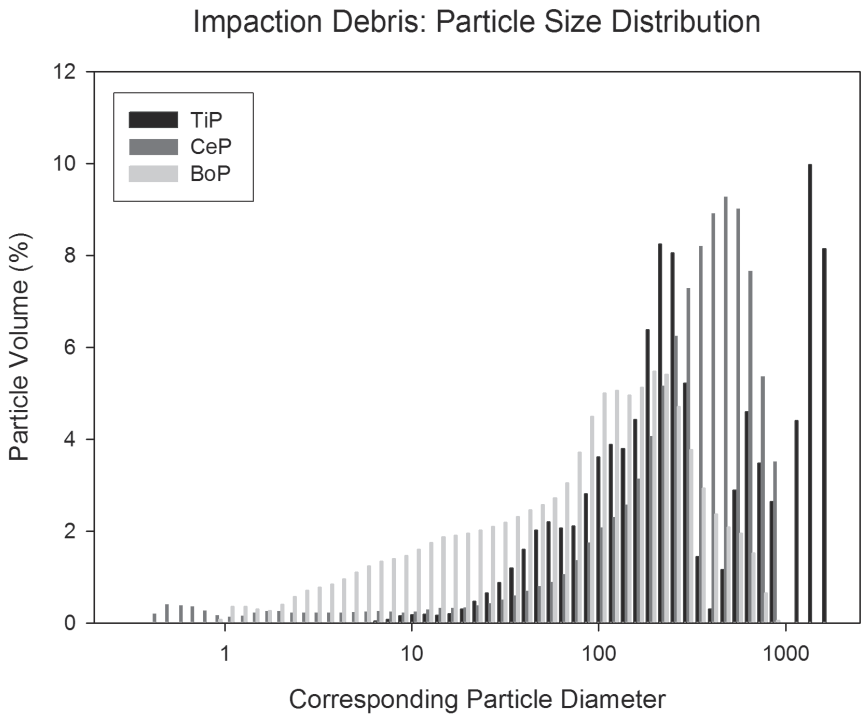


Figure 6 *Size distribution of particles generated during impaction (measured diameter range: 0.05 – 2,000 μm).*

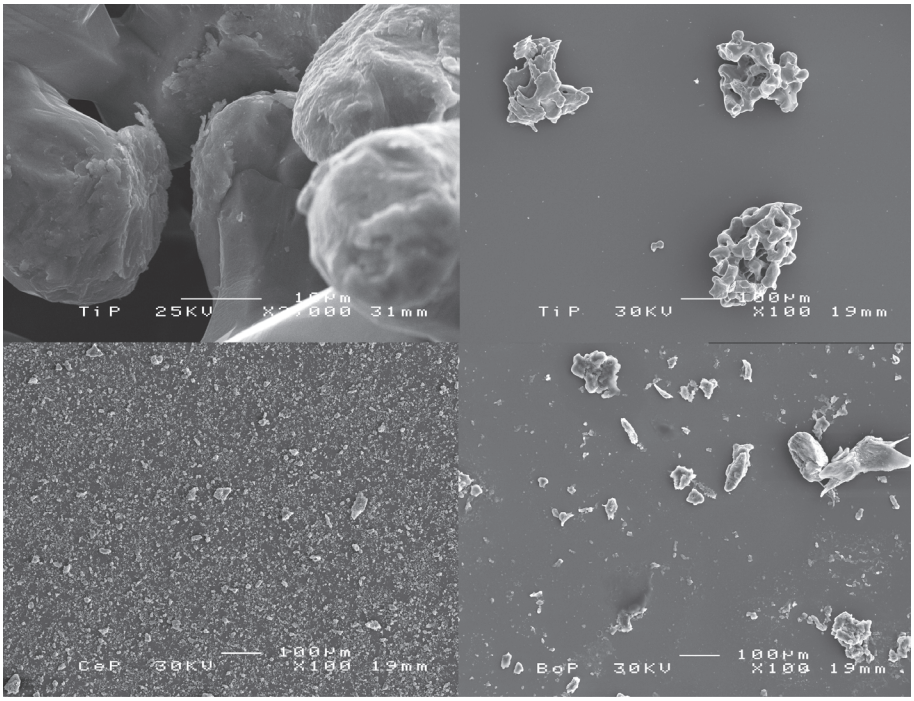


Figure 7 *Top left: particles < 10 μm consisted of flake like particles still attached to the surface of a TiP (2000x). Top right: TiP impaction debris consisted mainly of porous particles ≥ 100 μm (100x). Bottom shows sharp edged micro particles in a wide diameter range generated by CeP (left) and BoP (right).*

DISCUSSION

The disadvantages of allograft should be overcome by using a fully synthetic bone substitute that is available in large quantities. Like calciumphosphate, porous titanium in bulk application is an osteoconductive material [31,36]. It provides good bony anchorage after implantation which may be further enhanced by roughening or by application of a bioceramic coating [37-40]. Porous titanium particles which are impactible and adapt to defect geometry are a potential graft material for application in impaction grafting.

Obviously this study has some drawbacks. First, the large particle size of human grafts compared to the chamber (± 15 particles per specimen) and other tested particles could result in less efficient positioning within the testing chamber and inhomogeneous

specimens. However, variations in loading strain and stiffness mechanical parameters of all specimens were comparable to or smaller than after manually impacted of small bone chips indicating that the experiment set-up was adequate to test the grafts [17]. Second, it can be questioned whether the applied degree of impaction is comparable to that obtained during surgery. From a previous acetabular in vitro study in our own laboratory it was calculated that the applied amount of BoP was 1.4 – 1.5 g/ml graft layer [41]. Impacted fresh BoP specimens in this study had a bone graft density of 1.3 – 1.4 g/ml which seems similar. A further limitation was the use of different particle sizes of the various materials as this may affect the mechanical properties. In order to mimic realistic conditions with respect to current and potential future in vivo application of tested materials we deliberately have choosen to use different particle sizes for the tested materials. TiP were tested with a diameter range of 2 – 4 mm because this particle size can be applied in both smaller and larger defects. Large size CeP could not homogeneously be fitted in the test chamber and CeP suited for femoral application were tested. The effect of particle size on loading strain and stiffness of pure CeP seems to be relatively small [17,18,21,42]. Fourth, processed bone particles were used for generation and analysis of micro particles. This was done to prevent that generated micro particles sticked to the bone fat. Some protein denaturation or destruction can be expected from the described defatting and drying. Processed BoP seemed to be more brittle than fresh BoP. However, this was restored to a large extend by rehydration before impaction. The option of defattting after impaction was deliberately rejected to prevent the loss of impaction debris. Finally demineralized water was used instead of a more realistic fluid like a saline solution or blood. Demineralized water was choosen as a compromise to mimic the adhesive forces of a fluid film during impaction and to enable analysis of subsequently collected impaction debris.

The first question addressed in this study was whether TiP can be deformed under realistic impaction conditions and if a layer of impacted TiP maintains it's shape. Although constituted of pure metal, the compressibility of the highly porous titanium matrix was impressive. The combination of high impactability and firm entanglement seems promising for the application in impaction grafting: granules stick together well after only slight impaction and can be impacted further easily. Porosity of the titanium 'cookies' remained as high as 70 - 75% which is also a positive feature as there is ample inter-particle space

for tissue ingrowth. As opposed to TiP, individual bioceramic particles were damaged and actually pulvarized during impaction. Pulverization of porous bioceramic granules is also seen when bioceramic granules are applied in vitro en in vivo reconstructions [18,19,23]. Impaction of CeP resulted in a micro-porous graft layer constituted of small sticky particles rather than entangled ceramic particles. Although CeP showed higher impaction strains than BoP, their application in impaction grafting is associated with an increased peroperative fracture risk [20]. The ceramic granules behave like small brittle stones with sharp edges, resulting in high contact stresses when impacted against the surrounding bone tissue. Either the granule fragments or the surrounding bone tissue will be damaged. Porous titanium particles, however, are very ductile, have more rounded edges and can be gradually compressed. This will probably prevent the occurence of high contact stresses as seen during the application of CeP in impaction grafting.

Primary stability is a prerequisite to prevent early failure in orthopedic surgery and to allow ingrowth of vascularized tissue and especially bone. Therefore the graft layer should be resistant to both compression and shear stressess that take place during in vivo loading. Although shear resistance was not assessed in this study, impacted specimens were judged with respect to entanglement which influences shear resistance. From the observations we made of impacted specimens, we expect that a reconstructive layer made from the highly entangled, almost unified impacted TiP will be much more shear resistant than a reconstructive layer made of BoP or any mixture of BoP and CeP. With the confined compression test it was proven that impacted TiP were very resistant to compressive forces. Deformation of TiP during loading was very small and completely reversible during subsequent relaxation. TiP have the promising characteristic of combining impactability and stability after impaction. The dense CeP specimens were also very resistant to compression and showed only small plastic deformation after loading. The relatively large loading strain of CeP as reported by Verdonschot et al is probably the result of some crushing of CeP which were still intact after manual impaction [17].

As opposed to these synthetic materials, BoP showed a considerable amount of displacement during loading despite realistic impaction: during loading the impacted BoP specimens lost about 25 – 30% of their original height and only one third of this deformation was corrected during elastic recovery. Comparable observations were made after manually impaction of BoP [17). From these numbers it seems surprising

that impacted BoP result in clinically successful reconstructions [4,5]. Where plastic deformation imposes a threat on vitalization of the graft layer, axial micromotions during loading probably stimulate incorporation and ossification of bone grafts [43]. A high stiffness of the graft material prevents these micro motions and could prevent osseous differentiation of ingrowing tissue by stress shielding as it is known that some elastic deformation is beneficial for callus formation and callus mineralization [44,45].

Besides limiting bone ingrowth in the short term, the high stiffness of a non – resorbable matrix could jeopardize long-term quality of bony armouring. However, stress shielding is not expected in a reconstruction made with impacted TiP as the stiffness of the reconstructive TiP layer is iso-elastic compared to the trabecular bone in human vertebral bodies (209 ± 20 MPa and 251 ± 137 MPa respectively) [46]. Impacted TiP particles are about as stiff as a 50/50 weight mix of bone graft and BoneSave® [17]. Acetabular reconstructions made with a similar graft / BoneSave® mix (50/50 volume mix) showed good graft incorporation [23]. Moreover, the elastic deformation of TiP probably is about ten times as large as of porous tantalum (elastic modulus 3 GPa) which still shows good and long lasting bone ingrowth [47,49]. From these data it seems that the matrix formed by impacted TiP is flexible enough to allow transduction of loading forces sufficient for the formation and maintenance of bone tissue.

Another issue relevant to ingrowth of bone in TiP is the macro – and micro – porosity of a layer of impacted TiP. A study in goats with the bone conduction chamber showed that bone ingrowth in impacted, small and dense TiP was rather limited, probably by blocking of ingrowing bone and osteons due to the small pore sizes (10 – 50 µm) within the dense TiP graft cylinders. Complete filling of the TiP cylinders with fibrous tissue proved the interconnected porosity of TiP [50]. Consequently, TiP with a large pore size were selected for this mechanical study and further in vivo studies. These more porous TiP were used in a loaded in vivo pilot study in three goats with acetabular reconstruction of a large combined cavitary and segmental defect (type 3 AAOS defect), a cemented poly – ethylene cup and a cemented CoCr femoral prosthesis showed promising results. The porous reconstructive layer of TiP showed only limited cement penetration and direct contact between the reconstructive TiP layer and the surrounding acetabular bone. The reconstructive layer was locally invaded by new bone which was also in direct contact with the surface of the TiP (osseointegration) (non – published data).

One of the most important hazards threatening the long-term survival of orthopedic implants seems to be aseptic loosening by particle disease. Although titanium is considered to be a biocompatible material, particles of ''safe'' materials even like calciumphosphate inhibit osteoblast function and fibroblast function, stimulate osteolysis and cause cell death depending on particle size, exposed volume of particles, particle material and shape. Especially particles in the phagocytable range (<10 μm) are known to exhibit these negative effects [51-62]. Besides having a direct toxic effect, titanium particles generated during impaction and loading of TiP could enhance aseptic loosening by third body wear of other implanted parts. From this perspective, generated impaction debris for all three materials was quantified and analysed. TiP generated the smallest amount of ''impaction debris'' (0.2 weight %) and the smallest proportion of phagocytable particles (0.5 volume %). This corresponds to results of a study with realistic in vitro femoral reconstructions with 41 grams TiP with a structural and chemical composition identical to TiP used in this study. Reconstructions were made with commercially available femoral revision impaction tools and an Exeter™ hip prosthesis (Size 1; Stryker Orthopaedics) cemented in Sawbones femurs.The impaction debris amounted to 55 ± 18 mg (0.13 weight %) and prolonged loading did not seem to produce additional titanium micro particles [63]. In a spinal fusion pseudarthrosis study in rabbits as much as 190 mg phagocytable titanium debris had to applied in order to reduce the fusion rate by 20% [60]. Although the observed amount of titanium debris generated during impaction of TiP therefore appears rather small, it should be kept in mind that titanium particles are potentially more dangerous as they are non resorbable and probably more cytotoxic than calcium phosphate particles generated by CeP and BoP and the potential adverse effects of titanium micro particles generated during the impaction and loading of a reconstructive layer of TiP should be elucidated by in vivo studies. Although no signs of titanium particulate debris formation or osteolysis were found in the aforementioned loaded acetabular in vivo pilot study, the concentration of Ti in full blood samples showed a small but significant increase from 2.0 ± 2.2 ppb before operation to 4.3 ± 1.7 ppb ($p = 0.03$) twelve weeks after implantation of the TiP. Whether this is the result of an increasing contact area between the invading bone and the a stable TiP graft layer or an increased contact area as a result of fretting corrosion and titanium micro particle generation should be addressed in a definitive loaded in vivo study.

In conclusion porous titanium particles (TiP) can be impacted to a larger extent than bone grafts and create a highly entangled, macro porous graft layer. Impacted TiP are resistant to deformation by in vivo loading and are more elastic than bioceramic particles. The mechanical properties and the high porosity of impacted TiP together with the osteoconductive properties of titanium are promising characteristics with respect to ingrowth of fibrous tissue and bone. Moreover, TiP generated only a small amount of impaction debris compared bone and especially compared to bioceramic particles. Although this study indicates that TiP may be a promising bone graft substitute material, further in vitro and in vivo pre-clinical studies are warranted to assess clinical application and to elucidate the biological potential of TiP and the potential negative effects of debris particles.

Acknowledgements

This study was financed by Fondel Finance BV, Rotterdam, The Netherlands. Further, we thank Willem van de Wijdeven and Léon Driessen for their technical support.

REFERENCES

1. Fear J, Hillman M, Chamberlain M A, Tennant A. Prevalence of hip problems in the population aged 55 years and over: Access to specialist care and future demand for hip arthroplasty. British Journal of Rheumatology 1997; 36: 74-6.
2. Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. J Bone Joint Surg Am. 2005 Jul;87(7):1487-97.
3. Ong KL, Mowat FS, Chan N, Lau E, Halpern MT, Kurtz SM. Economic burden of revision hip and knee arthroplasty in Medicare enrollees. Clin Orthop Relat Res. 2006 May;446:22-8.
4. Schreurs B W, Busch V J J F, Welten M L, Verdonschot N, Slooff T J J H, Gardeniers J W M. Acetabular reconstruction with impaction bone-grafting and a cemented cup in patients younger than fifty years old. Journal of Bone and Joint Surgery-American Volume 2004; 86A: 2385-92.
5. Schreurs BW, Arts JJ, Verdonschot N, Buma P, Slooff TJ, Gardeniers JW. Femoral component revision with use of impaction bone-grafting and a cemented polished stem. J Bone Joint Surg Am. 2005 Nov;87(11):2499-507.
6. Galea G, Kopman D, Graham BJ. Supply and demand of bone allograft for revision hip surgery in Scotland. J Bone Joint Surg [Br] 1998;80-B:595–9.[
7. Conrad EU, Gretch DR, Obermeyer KR, et al. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg [Am] 1995;77-A:214–24.
8. Cook SD, Salkeld SL, Prewett AB. Simian immunodeficiency virus (human HIV-II) transmission in allograft bone procedures. Spine. 1995 Jun 15;20(12):1338-42.
9. Heekin RD, Engh CA, Vinh T. Morselized allograft in acetabular reconstruction. A postmortem retrieval analysis. Clin Orthop Relat Res. 1995 Oct;(319):184-90.
10. Nelissen RG, Bauer TW, Weidenhielm LR, LeGolvan DP, Mikhail WE. Revision hip arthroplasty with the use of cement and impaction grafting. Histological analysis of four cases. J Bone Joint Surg Am. 1995 Mar;77(3):412-22.
11. Buma P, Lamerigts N, Schreurs BW, Gardeniers J, Versleyen D, Slooff TJ. Impacted graft incorporation after cemented acetabular revision. Histological evaluation in 8 patients. Acta Orthop Scand. 1996 Dec;67(6):536-40.
12. Mann KA, Allen MJ, Ayers DC. Pre-yield and post-yield shear behavior of the cement-bone interface. J Orthop Res. 1998 May;16(3):370-8.
13. Johnson KD, Frierson KE, Keller TS, Cook C, Scheinberg R, Zerwekh J, Meyers L, Sciadini MF. Porous ceramics as bone graft substitutes in long bone defects: a biomechanical, histological, and radiographic analysis. J Orthop Res. 1996 May;14(3):351-69.
14. Rahimi F, Maurer BT, Enzweiler MG. Coralline hydroxyapatite: a bone graft alternative in foot and ankle surgery. J Foot Ankle Surg. 1997 May-Jun;36(3):192-203; discussion 255-6.
15. Delecrin J, Takahashi S, Gouin F, Passuti N. A synthetic porous ceramic as a bone graft substitute in the surgical management of scoliosis: a prospective, randomized study. Spine. 2000 Mar 1;25(5):563-9.
16. Oonishi H, Kadoya Y, Iwaki H, Kin N. Total hip arthroplasty with a modified cementing technique using hydroxyapatite granules. J Arthroplasty. 2001 Sep;16(6):784-9.
17. Verdonschot N, van Hal CT, Schreurs BW, Buma P, Huiskes R, Slooff TJ. Time-dependent mechanical properties of HA/TCP particles in relation to morsellized bone grafts for use in impaction grafting. J Biomed Mater Res. 2001;58(5):599-604.

18. Bolder SB, Verdonschot N, Schreurs BW, Buma P. The initial stability of cemented acetabular cups can be augmented by mixing morsellized bone grafts with tricalciumphosphate/ hydroxyapatite particles in bone impaction grafting. J Arthroplasty. 2003 Dec;18(8):1056-63.
19. Arts JJ, Schreurs BW, 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 Mech Eng [H]. 2005 Jul;219(4):257-63.
20. van Haaren EH, Smit TH, Phipps K, Wuisman PI, Blunn G, Heyligers IC. 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 Feb;87(2):267-71.
21. Blom AW, Grimm B, Miles AW, Cunningham JL, Learmonth ID. Subsidence in impaction grafting: the effect of adding a ceramic bone graft extender to bone. Proc Inst Mech Eng [H]. 2002;216(4):265-70.
22. Blom AW, Cunningham JL, Hughes G, Lawes TJ, Smith N, Blunn G, Learmonth ID, Goodship AE. The compatibility of ceramic bone graft substitutes as allograft extenders for use in impaction grafting of the femur. J Bone Joint Surg Br. 2005 Mar;87(3):421-5.
23. Arts JJ, Gardeniers JW, Welten ML, Verdonschot N, Schreurs BW, Buma P. No negative effects of bone impaction grafting with bone and ceramic mixtures. Clin Orthop Relat Res. 2005 Sep;438:239-47.
24. Hing KA, Wilson LF, Buckland T. Comparative performance of three ceramic bone graft substitutes. Spine J. 2007 Jul-Aug;7(4):475-90.
25. Bloebaum RD, Rhodes DM, Rubman MH, Hofmann AA.. Bilateral tibial components of different cementless designs and materials. Microradiographic, backscattered imaging, and histologic analysis. Clin Orthop Relat Res. 1991 Jul;(268):179-87.
26. Vervest TM, Anderson PG, Van Hout F, Wapstra FH, Louwerse RT, Koetsier JW. Ten to twelve-year results with the Zweymuller cementless total hip prosthesis. J Arthroplasty. 2005 Apr;20(3):362-8.
27. Meyer U, Joos U, Mythili J, Stamm T, Hohoff A, Fillies T, Stratmann U, Wiesmann HP. Ultrastructural characterization of the implant/bone interface of immediately loaded dental implants. Biomaterials. 2004 May;25(10):1959-67.
28. Zwipp H, Rammelt S, Barthel S. Calcaneal fractures--open reduction and internal fixation (ORIF). Injury. 2004 Sep;35 Suppl 2:SB46-54.
29. Sun C, Huang G, Christensen FB, Dalstra M, Overgaard S, Bunger C. Mechanical and histological analysis of bone-pedicle screw interface in vivo: titanium versus stainless steel. Chin Med J (Engl). 1999 May;112(5):456-60.
30. Christensen FB, Dalstra M, Sejling F, Overgaard S, Bunger C. Titanium-alloy enhances bone-pedicle screw fixation: mechanical and histomorphometrical results of titanium-alloy versus stainless steel. Eur Spine J. 2000 Apr;9(2):97-103.
31. Voor MJ, Arts JJ, Klein SA, Walschot LH, Verdonschot N, Buma P. Is hydroxyapatite cement an alternative for allograft bone chips in bone grafting procedures? A mechanical and histological study in a rabbit cancellous bone defect model. J Biomed Mater Res B Appl Biomater. 2004 Nov 15;71(2):398-407.
32. Bolder SB, Schreurs BW, Verdonschot N, van Unen JM, Gardeniers JW, Slooff TJ. Particle size of bone graft and method of impaction affect initial stability of cemented cups: human cadaveric and synthetic pelvic specimen studies. Acta Orthop Scand. 2003 Dec;74(6):652-7.

33. Dunlop DG, Brewster NT, Madabhushi SP, Usmani AS, Pankaj P, Howie CR. Techniques to improve the shear strength of impacted bone graft: the effect of particle size and washing of the graft. J Bone Joint Surg Am. 2003 Apr;85-A(4):639-46.

34. Dalstra M. Differences in pelvic load transfer due to variations in acetabular cup design. Thesis, printed by Leijn, Nijmegen, The Netherlands, 1993.

35. Weinans H, Huiskes R, Grootenboer HJ. Effects of material properties of femoral hip components on bone remodeling. J Orthop Res. 1992 Nov;10(6):845-53.

36. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. Biomaterials. 2005 Oct;26(30):6014-23.

37. Gotfredsen K, Berglundh T, Lindhe J. Anchorage of titanium implants with different surface characteristics: an experimental study in rabbits. Clin Implant Dent Relat Res. 2000;2(3):120-8.

38. Grizon F, Aguado E, Hure G, Basle MF, Chappard D. Enhanced bone integration of implants with increased surface roughness: a long term study in the sheep. J Dent. 2002 Jul-Aug;30(5-6):195-203.

39. Ramires PA, Wennerberg A, Johansson CB, Cosentino F, Tundo S, Milella E. Biological behavior of sol-gel coated dental implants. J Mater Sci Mater Med. 2003 Jun;14(6):539-45.

40. Park YS, Yi KY, Lee IS, Han CH, Jung YC. The effects of ion beam-assisted deposition of hydroxyapatite on the grit-blasted surface of endosseous implants in rabbit tibiae. Int J Oral Maxillofac Implants. 2005 Jan-Feb;20(1):31-8.

41. Walschot LH, Schreurs BW, Buma P, R. Aquarius, Verdonschot N. Porous titanium particles for application in impaction grafting: mechanical stability and cement penetration in cavitary acetabular reconstructions. Poster number 1671. Annual Meeting of the Orthopaedic Research Society 2008, San Fransisco, CA, USA.

42. Arts JJ, Verdonschot N, Buma P, Schreurs BW. Larger bone graft size and washing of bone grafts prior to impaction enhances the initial stability of cemented cups: experiments using a synthetic acetabular model. Acta Orthop. 2006 Apr;77(2):227-33.

43. Lamerigts NM, Buma P, Huiskes R, Schreurs W, Gardeniers J, Slooff TJ. Incorporation of morsellized bone graft under controlled loading conditions. A new animal model in the goat. Biomaterials. 2000 Apr;21(7):741-7.

44. Goodship AE, Kenwright J. The influence of induced micromovement on the healing of experimental tibial fractures. J Bone Joint Surg Br. 1985;67:650-5.

45. Claes LE, Wilke HJ, Augat P, Rübenacker S, Margevicius KJ. Effect of dynamization on gap healing of diaphyseal fractures under external fixation. Clin Biomech (Bristol, Avon). 1995;10:227-34.

46. Rapillard L, Charlebois M, Zysset PK. Compressive fatigue behavior of human vertebral trabecular bone. J Biomech. 2006;39(11):2133-9. Epub 2005 Jul 26.

47. Bobyn JD, Stackpool GJ, Hacking SA, Tanzer M, Krygier JJ. Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. J Bone Joint Surg Br. 1999 Sep;81(5):907-14.

48. Bobyn JD, Toh KK, Hacking SA, Tanzer M, Krygier JJ. Tissue response to porous tantalum acetabular cups: a canine model. J Arthroplasty. 1999 Apr;14(3):347-54.

49. Unger AS, Lewis RJ, Gruen T. Evaluation of a porous tantalum uncemented acetabular cup in revision total hip arthroplasty: clinical and radiological results of 60 hips.J Arthroplasty. 2005 Dec;20(8):1002-9.

50. Walschot LHB, Verdonschot N, Schreurs BW, Buma P. Bone ingrowth in porous titanium particles and allografts: a bone chamber study. Poster number 1620. Annual Meeting of the Orthopaedic Research Society 2007, San Diego, CA, USA.

51. Bi Y, Van De Motter RR, Ragab AA, Goldberg VM, Anderson JM, Greenfield EM. Titanium particles stimulate bone resorption by inducing differentiation of murine osteoclasts. J Bone Joint Surg Am. 2001 Apr;83-A(4):501-8.

52. Wang JC, Yu WD, Sandhu HS, Betts F, Bhuta S, Delamarter RB. Metal debris from titanium spinal implants. Spine (Phila Pa 1976). 1999 May 1;24(9):899-903.

53. Wang ML, Tuli R, Manner PA, Sharkey PF, Hall DJ, Tuan RS. Direct and indirect induction of apoptosis in human mesenchymal stem cells in response to titanium particles. J Orthop Res. 2003 Jul;21(4):697-707.

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

55. Matthews JB, Green TR, Stone MH, Wroblewski BM, Fisher J, Ingham E Comparison of the response of three human monocytic cell lines to challenge with polyethylene particles of known size and dose.. J Mater Sci Mater Med. 2001 Mar;12(3):249-58.

56. Zysk SP, Gebhard HH, Kalteis T, Schmitt-Sody M, Jansson V, Messmer K, Veihelmann A. Particles of all sizes provoke inflammatory responses in vivo. Clin Orthop Relat Res. 2005 Apr;(433):258-64.

57. Sieving A, Wu B, Mayton L, Nasser S, Wooley PH. Morphological characteristics of total joint arthroplasty-derived ultra-high molecular weight polyethylene (UHMWPE) wear debris that provoke inflammation in a murine model of inflammation. J Biomed Mater Res A. 2003 Mar 1;64(3):457-64.

58. Ingram JH, Stone M, Fisher J, Ingham E. The influence of molecular weight, crosslinking and counterface roughness on TNF-alpha production by macrophages in response to ultra high molecular weight polyethylene particles. Biomaterials. 2004 Aug;25(17):3511-22.

59. von Knoch M, Jewison DE, Sibonga JD, Sprecher C, Morrey BF, Loer F, Berry DJ, Scully SP The effectiveness of polyethylene versus titanium particles in inducing osteolysis in vivo. J Orthop Res. 2004 Mar;22(2):237-43.

60. Cunningham BW, Orbegoso CM, Dmitriev AE, Hallab NJ, Sefter JC, McAfee PC. The effect of titanium particulate on development and maintenance of a posterolateral spinal arthrodesis: an in vivo rabbit model. Spine (Phila Pa 1976). 2002 Sep 15;27(18):1971-81.

61. Mostardi RA, Meerbaum SO, Kovacik MW, Gradisar IA Jr. Response of human fibroblasts to tantalum and titanium in cell culture. Biomed Sci Instrum. 1997;33:514-8.

62. Biomaterials. 2000 Jun;21(11):1103-14. The effects of calcium phosphate cement particles on osteoblast functions. Pioletti DP, Takei H, Lin T, Van Landuyt P, Ma QJ, Kwon SY, Sung KL.

63. Aquarius R, Walschot L, Buma P, Schreurs BW, Verdonschot N. Clin Orthop Relat Res. 2009 Jun;467(6):1538-45. Epub 2009 Jan 13. In vitro testing of femoral impaction grafting with porous titanium particles: a pilot study.

CHAPTER THREE

IN VITRO TESTING OF FEMORAL IMPACTION GRAFTING WITH POROUS TITANIUM PARTICLES: A PILOT STUDY

Clin Orthop Relat Res 2009;467(6):1538-45.

ABSTRACT

The disadvantages of allografts to restore femoral bone defects during revision hip surgery have led to the search for alternative materials. We investigated the feasibility of using porous titanium particles and postulated the following questions: (1) Is it possible to create a high-quality femoral graft of porous TiP in terms of graft thickness, cement thickness, and cement penetration? (2) Does this TiP graft layer provide initial stability when a femoral cemented stem is implanted in it? (3) What sizes of particles are released from the porous titanium particles during impaction and subsequent cyclic loading of the reconstruction? We simulated cemented revision reconstructions with porous titanium particles in seven composite femurs loaded for 300,000 cycles and measured stem subsidence. Particle release from the titanium particle grafts was analyzed during impaction and loading. Impacted titanium particles formed a highly interlocked graft layer. We observed limited cement penetration into the titanium particle graft. A total mean subsidence of 1.04 mm was observed after 300,000 cycles. Most particles released during impaction were in the phagocytable range (< 10 µm). There was no detectable particle release during loading. Based on the data, we believe titanium particles are a promising alternative for allografts. However, animal testing is warranted to investigate the biologic effect of micro particle release.

INTRODUCTION

The aging of the population and the tendency to treat younger patients with various hip disorders has increased the incidence of primary and revision THAs in Western countries over the past 20 years [16,24,35]. Annually, more than 40,000 revision procedures are performed on patients in the United States alone [21]. A major problem of revision surgery is the loss of bone stock which compromises implantation of a new prosthesis [16,18]. Morselized allografts, in combination with the bone impaction grafting technique, can restore the bone stock [14,28]. The impacted bone restores short-term stability for the revision prosthesis and allows long-term restoration of bone stock [17,36].
Allografts, however, have important disadvantages such as risk of viral transmission, infection, and limited availability [8,9,13,25,33,39]. Because of these drawbacks, various studies have been performed to identify suitable materials to replace bone grafts, such as tricalcium phosphate and hydroxyapatite [2,5,32]. Granules made of these materials are used as a bone graft extender, thereby reducing the amount of bone grafts necessary for bone impaction grafting. However, these materials cannot fully replace bone grafts because calcium phosphate particles can pulverize during the impaction phase when used as a stand-alone replacement for bone grafts [2,4,5]. Other materials thus are required to replace bone chips in revision hip surgery. Any new material that will be used to replace bone grafts should meet certain criteria. The material should be biocompatible and suitable for impaction, and the impacted mass should create a stable environment for a prosthesis. Cement penetration into the graft material should be limited because excessive cement penetration would obstruct bone ingrowth into the graft material.

As an alternative material, we propose porous titanium particles, a nonresorbable replacement for the morselized cancellous bone grafts. Titanium is a biocompatible material often used in orthopaedic and dental implants [1,22]. In addition to the biocompatibility, titanium has osteoinductive characteristics when implanted in bulk amounts in an organism [12,40]. A disadvantage of porous TiP is possible release of small wear particles (< 10 μm) during impaction and cyclic loading. This can be considered a potential danger because particulate debris can induce osteolysis [20,31,41,45,47].

Contrary to the biologic response, nothing is known about the handling properties of titanium particles as an impaction grafting material and the possible stability they can provide for a prosthesis. Even though there is no experience with this material, stem stability should be comparable or better than stems implanted with morselized allograft because massive early subsidence can indicate failure of a reconstruction [11].
We therefore addressed the following questions: (1) Is it possible to create a high-quality femoral graft of porous titanium particles providing adequate graft thickness, cement thickness, and cement penetration? (2) Does this titanium particle graft layer provide initial stability when a femoral cemented stem is implanted in it? (3) What sizes of particles are released from the porous titanium particles during impaction and after subsequent loading of the impacted titanium particle layer?

MATERIALS AND METHODS

We simulated seven femoral revision reconstructions with a graft of the experimental titanium particles. Before and during the 300,000 loading cycles that were applied to all revision reconstructions, stem subsidence (mm) was measured using roentgen stereophotogrammetric analysis (RSA) and particle generation (presented in volume percentages) was measured using the laser diffraction technique. After loading, the reconstructions were sectioned in the frontal plane and the cement mantle thickness, cement penetration, and titanium graft thickness were measured (mm).

We used seven large, left third-generation composite femurs (Part Number 3306, Sawbones®; Pacific Research Laboratories Inc, Malmö, Sweden) for the experiment. All models were transversely sectioned at the diaphysis 26 cm distal to the tip of the greater trochanter. Similar to the study by Barker et al [3], we widened the medullary canal to a diameter of 18 mm (initial diameter, 16 mm) to simulate cortical thinning as seen in revision surgery. Proximally located polyurethane, representing trabecular bone, was partly removed using various broaches. As in our clinical protocol, the first step in the revision operation was to place a plug in the distal end of the medullary canal. The plug had two major purposes: to facilitate placement of a central guidewire and to limit the titanium particle graft distally.

The titanium particles were produced during the purification of titanium through titanium tetrachloride. This process creates porous commercially pure titanium with a crystalline microtexture for supplying the titanium particles. Two sizes of particles were used, referred to as either small or large particles. The small particles had a diameter in the range of 2.8 to 3.2 mm (ie, passed a 3.2-mm pore sieve but stopped by a 2.8-mm pore sieve) and the larger particles had a diameter of 3.2 to 4.0 mm (ie, passed a 4.0-mm pore sieve but stopped by a 3.2-mm pore sieve). After sieving, the titanium particles were subjected to standardized cleaning to remove potential particulates and chemical contamination (procedure number PS03-016; CAM Implants BV, Leiden, The Netherlands). From cross-sectional photographs (Jeol 6310 scanning electron microscope [SEM]; JEOL Ltd, Tokyo, Japan), we calculated the porosity of the titanium particles to be 83 ± 2% (mean ± standard deviation [SD]); pores were interconnected.

The graft layer, which comprised a volume of 37 mL, was carefully constructed in several layers, similar to clinical practice with allografts. Every layer was axially impacted followed by impaction in a radial fashion using tapered impactors to ensure optimal compression of the titanium particles. We performed all impaction steps with the X-Change® revision instrumentation up to phantom Size 1 (Stryker Orthopaedics, Mahwah, NJ). Two different titanium particle sizes were used: 64 mL of large titanium particles (diameter, 3.2-4.0 mm) to fill larger spaces in mostly the proximal and distal areas of the Sawbones® femurs and 18 mL of the smaller titanium particles (diameter, 2.8-3.2 mm) to create the graft layer in the middle part of the reconstruction. Before impaction, the TiP was soaked in distilled water to improve handling.

After the complete titanium graft layer was constructed, an Exeter™ hip prosthesis (Size 1; Stryker Orthopaedics) was cemented in the Sawbones® femurs. The cement was allowed to polymerize at room temperature for at least 48 hours. Stem migration on loading was quantified with RSA; we glued six tantalum pellets to the head of the stem in addition to a lead pellet already attached to the tip of the prosthesis. An Exeter™ stem centralizer was used to prevent the lead pellet from being detached from the tip of the prosthesis by the cement during insertion of the stem. Another 12 tantalum pellets were attached to the lateral and medial sides of the Sawbones® femurs.

After attachment of the tantalum pellets, we placed the Sawbones® femurs in 7° lateral tilt in a custom-made metal support. The metal support was coupled to a polysulfone

filter casing (Part Number 10461000; Whatman Schleicher & Schuell, 's-Hertogenbosch, The Netherlands) that contained an exchangeable cellulose acetate membrane filter (Part Number 10404006; Whatman Schleicher & Schuell). The filters had a pore size of 0.45 µm, so small titanium debris could still be captured. We placed a polymethylmethacrylate reservoir that completely surrounded the Sawbones® femur on the metal support. On top of the polymethylmethacrylate reservoir was a polyethylene lid that sealed the experimental setup (Figure 1).

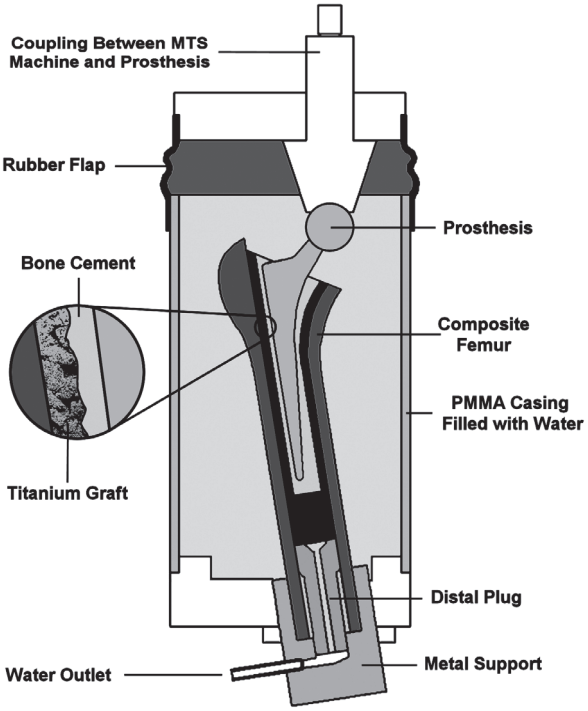


Figure 1 *A diagram provides an overview of the experimental setup. PMMA = polymethylmethacrylate. See page 196 for color figure.*

The experimental setup then was filled with distilled water, submerging the entire Sawbones® femur, and placed under a servohydraulic MTS® machine (MTS Systems Corp, Eden Prairie, MN). The reconstruction was subjected to a dynamic, axial load of 20 to 3000 N for 300,000 consecutive loading cycles at 2 Hz. To analyze stem subsidence with respect

to the Sawbones® femurs, we performed RSA measurements under loaded conditions (3000 N) after 0, 1000, 10,000, 50,000, 150,000, and 300,000 cycles. Displacement of the stem in the distal direction was defined as negative subsidence. Unloaded RSA measurements also were performed at the beginning (0 cycles) and at the end (300,000 cycles) of the experiment. In addition to every RSA measurement at 0, 1000, 10,000, 50,000, 150,000, and 300,000 cycles, 200 mL distilled water was forced through the cellulose acetate filter. With a syringe connected to the distal end of the filter casing, we could suck the distilled water through the cellulose acetate filter and thereby also force it to travel through the entire graft layer. A new filter was used for every 100 mL distilled water going through the reconstruction. The measurement at 0 cycles would give us an idea about the particles released by impaction; the rest of the measurements would provide more information on possible particle release resulting from dynamic loading. All samples were analyzed using a laser diffraction particle sizer (Malvern Mastersizer® 2000; Malvern Instruments Ltd, Worcestershire, UK).

Directly after completion of the impaction phase of the first reconstruction (of seven), the central guiding rod was retracted to proceed with the cementing phase. However, the remainder of the water used to soak the TiP would run out of the reconstruction. Because this remaining 'soaking water' was possibly filled with titanium particles, we decided to collect it in plastic containers in the last six reconstructions and analyze these samples using the laser diffraction technique to measure the size of possible particles. The 'soaking water' was centrifuged to make sure the measured titanium particles were not contaminated with polyurethane from the composite femur. In addition, we also quantified the weight of the titanium debris.

After the 300,000 loading cycles, the Sawbones® femurs were sectioned in the frontal plane and the sections scanned under a GT-12000 flatbed scanner (Epson, Amsterdam, The Netherlands). These scans then were analyzed with analySIS® AUTO 3.2 (Soft Imaging System GmbH, Münster, Germany). This software allows the user to measure distances on digital photographs or scans. In this way, we were able to measure graft thickness and cement mantle thickness at 5-mm intervals along the whole reconstruction. Cement protruding into the titanium particle graft was defined as cement penetration. This was also determined from the scans. Because cement penetration into the titanium particle graft was not seen everywhere along the titanium, we calculated this by subtracting the average cement mantle thickness from the average maximal cement thickness.

To analyze whether the cement and the titanium particle graft were evenly distributed, we determined if there were any differences in the reconstructions between the medial and lateral (1) graft layer, (2) cement mantle and (3) cement penetration in the titanium particle graft. Therefore we have used the measurements of the scanned reconstructions and applied a Student's t-test to detect any statistically differences between the medial and lateral sides of the reconstructions. The tests were performed with Microsoft® Excel® 2007 (Microsoft, Redmond, WA).

RESULTS

It was possible to construct a graft layer of impacted TiP in the femurs using the femoral bone impaction system. The porous titanium particles were heavily interlocked and formed a porous structure (Figure 2). All specimens showed reproducible impaction of the titanium particles resulting in a firm titanium graft with an average thickness of 3.53 mm (SD, 1.43 mm) (Table 1).

Specimen	Cement layer (mm)	Graft layer (mm)	Cement penetration (mm)
1	2.17 (0.69)	3.63 (1.47)	0.65
2	2.52 (1.08)	3.43 (1.46)	0.47
3	2.45 (0.54)	3.57 (1.56)	0.35
4	2.23 (0.98)	3.47 (1.25)	0.50

Table 1 *Measurement results. Values are expressed as averages, with standard deviations in parentheses.*

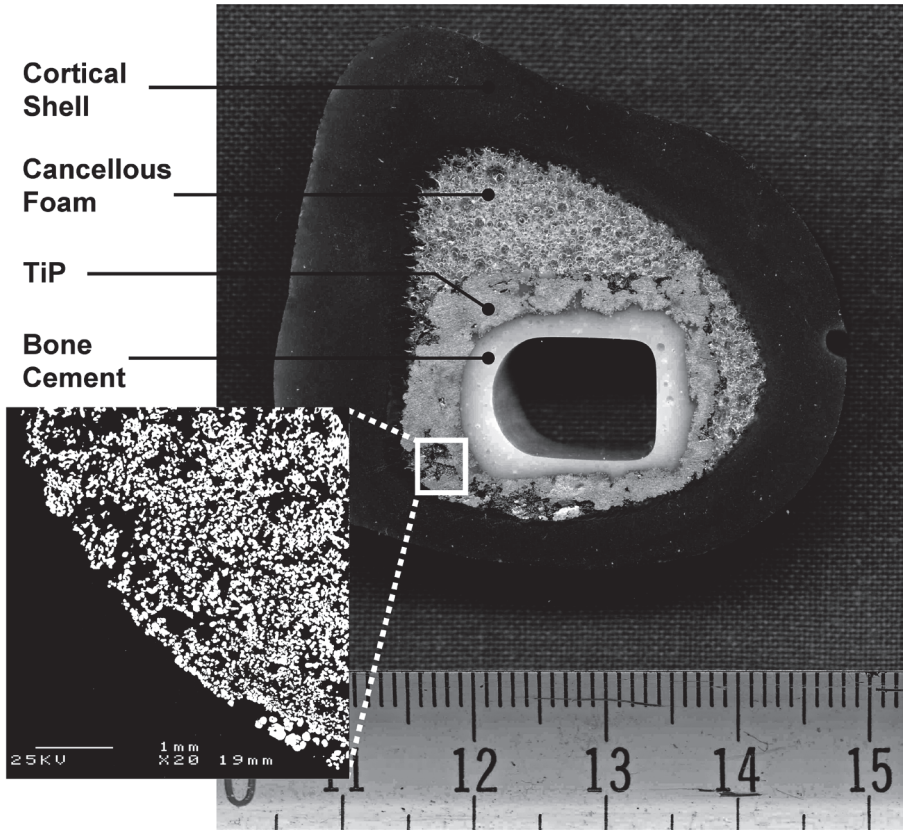


Figure 2 *A proximal transverse cross section with closeup shows a highly porous graft layer (backscatter SEM at 25 kV, 20x magnified). TIP = titanium particles. See page 197 for color figure.*

The bone cement had an average thickness of 2.34 mm (SD, 0.86 mm). The average cement penetration into the TiP graft was 0.49 mm (SD, 0.11 mm). There was a different cement thickness between the medial and lateral side of the cement mantle. With 1.82 mm (SD, 0.76 mm), the medial cement mantle was thinner ($p < 0.001$) than the lateral side of the cement mantle, which was 2.89 mm (SD, 0.54 mm) (Figure 3). Two Sawbone femurs failed during cyclic loading. This failure was not related to the titanium particle graft or the proximomedial fissure that had occurred in one of the femurs during impaction, but attributable to damage to the distal part of the composite femur that was caused by the clamps during impaction. These two reconstructions could no longer be used for graft or

cement measurements. A third reconstruction was sectioned into transverse slices for visual inspection of the reconstruction (Figure 2).

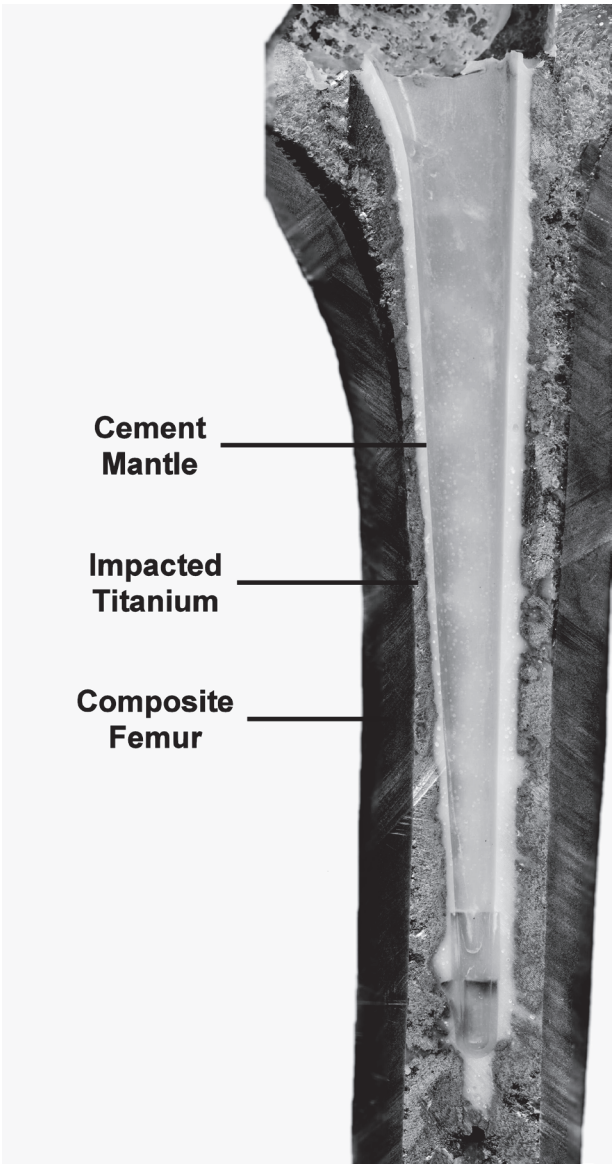


Figure 3 *A frontal section of one of the reconstructions shows the titanium graft layer, cement mantle, and cement penetration. The cement mantle on the medial side (left; 1.82 mm) is thinner ($p < 0.001$) than the cement mantle on the lateral side (right; 2.89 mm). The titanium graft is a porous structure but seems massive as an artifact of sectioning.* *See page 198 for color figure.*

The reconstructions were stable. When the load was applied to the revision reconstructions for the first time, settling of the stem into the graft layer was seen. This resulted in an average initial subsidence of 0.59 mm (SD, 0.41 mm). After this initial settling, a low and reproducible time-dependent subsidence of the stem relative to the bone was observed during cyclic loading. After 300,000 loading cycles, time-dependent stem subsidence was 0.45 mm (SD, 0.04 mm) for the five remaining stems (Figure 4). A typical fast initial stem subsidence rate, which gradually diminished toward the end of the experiment, was observed (Figure 4). The last subsidence measurements of the two failed reconstructions were -0.61 mm after 150,000 loading cycles (Femur 6; failure at 266,000 cycles) and -0.28 mm at 50,000 loading cycles (Femur 7; failure at 130,000 cycles). A proximomedial fissure occurred during impaction of the titanium particles in Femur 6. Despite the cerclage wire we used to repair the femur, above-average subsidence of the stem still was observed. The average total subsidence, comprising both initial settling and time-dependent subsidence, after 300,000 loading cycles was 1.04 mm (SD, 0.41 mm) for the five remaining stems.

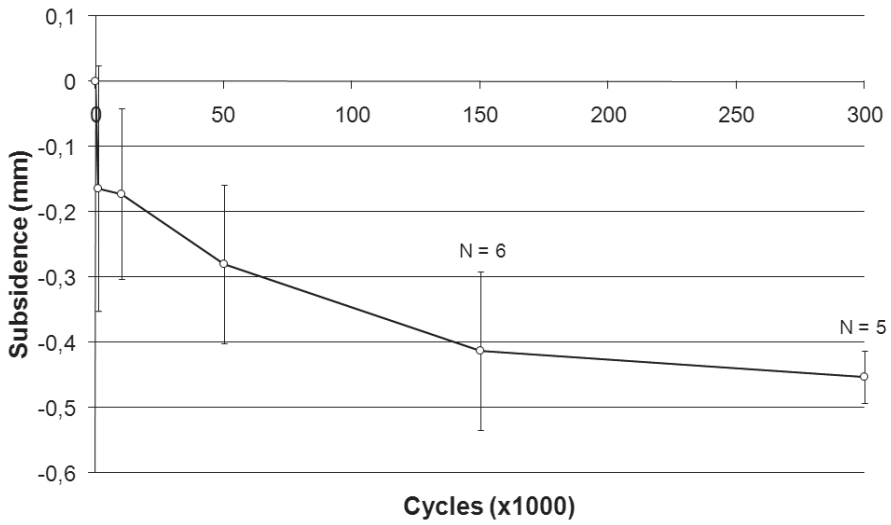


Figure 4 *A graph shows the average time-dependent subsidence of the seven Exeter™ stems. Two femurs failed before the end of the test: one before t = 150,000 cycles and the other before t = 300,000. After the initial settling of 0.59 mm (not shown in graph), a time-dependent stem subsidence of 0.45 mm for the five remaining stems was observed after 300,000 loading cycles.*

Titanium particles were released during impaction of the TiP. The average amount of titanium debris collected after impaction was 55 mg (SD, 18 mg). A 0.32 volume percent of the particles had a diameter equal or smaller than 10.48 µm (the size of particles that can be phagocytized by cells). The bulk of the weight (> 99 volume percent) comprised particles with a diameter larger than 10.48 µm (Figure 5). However, the majority of the particles (> 99%) had a diameter of 10.48 µm or smaller because small particles take up less volume. The filters used to collect particles released during dynamic loading contained too few particles to be analyzed by the laser diffraction method.

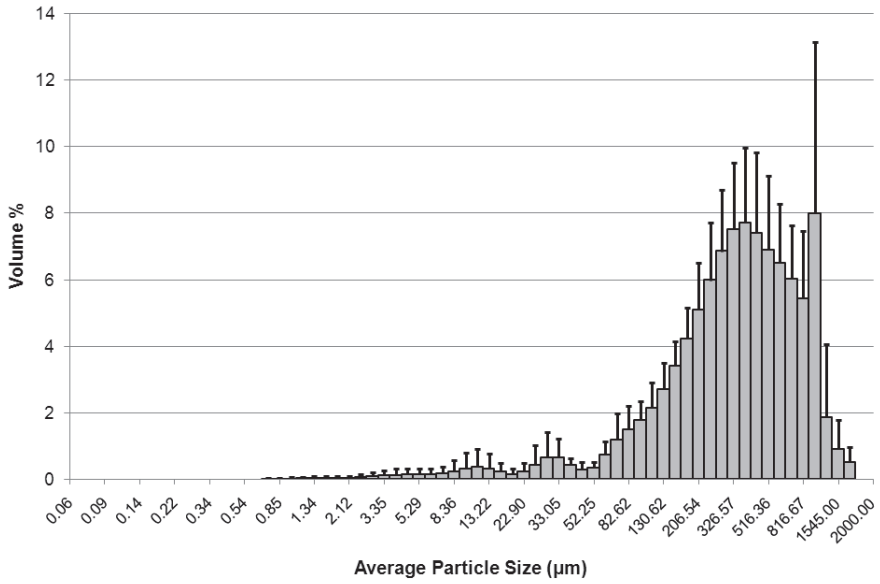


Figure 5 *A graph shows the average volume percent per particle size category found in the soaking water directly after impaction of the graft in the last six reconstructions. A 0.32-volume percent of the particles had a diameter of 10.48 µm or smaller (the size of particles that can be phagocytized by cells). The bulk of the weight (> 99 volume percent) comprised particles with a diameter larger than 10.48 µm. However, the majority of the particles (> 99%) had a diameter of 10.48 µm or smaller because small particles take up less volume.*

DISCUSSION

The disadvantages of allografts, such as shortage, infection, and risks of viral transmission, have led to a search for alternative materials in bone impaction grafting [8,9,13,25,33,39]. We therefore evaluated a new possible bone graft substitute, titanium particles. We specifically addressed the following questions: (1) Is it possible to create a high-quality femoral graft of porous titanium particles in terms of graft thickness, cement thickness, and cement penetration? (2) Does this titanium particle graft layer provide initial stability when a femoral cemented stem is implanted in it? (3) What sizes of particles are released from the porous titanium particles during impaction and after subsequent loading of the titanium particle layer?

This study has some limitations. First, because of the use of a composite femur model, morphologic variability as encountered in the operating room is absent. However, the synthetic femurs increase reproducibility and precision of the experiment. Second, the composite femur is comparable to bone of younger people [34]. We believe, by simulating cortical thinning, the bones are more comparable to (osteoporotic) bones that are typically seen in revision surgery. Third, no control revision reconstructions with morselized cancellous bone grafts were made for comparison of stem subsidence. However, the literature provided data obtained in ways similar to those in our experiment. Also, the strenuous loading protocol we used provided a safety margin when our subsidence values were compared with those from published studies. Fourth, two Sawbones® femurs of the experiment broke before the end of the testing protocol. Because the fractures originated at the location of clamping during the impaction procedure, we believe stress peaks in the synthetic bone must have damaged the bone, which eventually initiated a fatigue fracture in the femur. There was no relationship between the failure of the femurs and a proximomedial fissure that was present in one of the failed reconstructions. Therefore, these fractures should be considered an experimental artifact attributable to the clamping during impaction and not linked to the titanium particle graft. Finally, the laser diffraction method has limitations. In this device, particles pass a laser beam and thereby scatter the light. This scattered light, which is typical for each size, is then caught by a sensor. The method assumes all particles to be spheres. Titanium particles, however, can have shapes ranging from sphere-like to rod-like [37]. After careful SEM analyses of

several filters obtained in this study, we found, although some particles were rod-shaped, most were sphere-shaped. Therefore, we believe errors in our measurements do not greatly affect the outcome of this study.

We considered the obtained characteristics of the reconstructions adequate. A highly interlocked titanium layer graft layer was established around the whole prosthesis (Figure 2). The thickness of this layer was similar to the reported thickness of impacted bone chips [3,42]. Previous reports suggested some parts of the cement mantles in revision surgery with impacted allografts were absent or less than 2 mm.[26,27] None of the four specimens we examined showed absence of the cement mantle in any region. However, the cement mantle on the medial side (especially Gruen Zones 5 and 6) was considerably thinner than the mantle on the lateral side of the sections. The average cement penetration was very small, which is a positive finding because excessive cement penetration can hamper bone ingrowth into the titanium graft layer. The average cement penetration was only 0.49 mm, indicating the reconstruction obtains its stability by the granules and not by excessive cement penetration as reported previously in a study with calcium phosphate particles [4].

The distal migration of the Exeter™ stem relative to the Sawbones® femurs was in line with other in vitro studies. In two recent studies similar to our experiment, Exeter™ stems were used in combination with the impaction grafting technique [30,44]. In one study, subsidence values of 0.44 mm and 0.13 mm were observed after 18,000 cycles for reconstructions with only bone chips and reconstructions that contained allografts and hydroxyapatite graft extenders, respectively [30]. The other study had subsidence values of 2.31 mm and 0.99 mm after 10,000 loading cycles for reconstructions with pure allografts and reconstructions with a mix of allografts and hydroxyapatite graft extenders, respectively [44]. Our average total subsidence after 10,000 and 50,000 loading cycles was 0.76 mm (SD, 0.43 mm) and 0.87 (SD, 0.43 mm), respectively. This is in line with published studies, especially when our strenuous loading regime is considered. Perhaps the most important finding is that the time-dependent stem subsidence rate diminishes toward the end of the experiment, suggesting a stable situation. When we compare our findings with those of in vivo experiments, the 300,000 load cycles correspond with 3 months of normal loading for an active patient.[29] With this in mind, our results were in line with subsidence of an Exeter™ stem in combination with morselized allografts

[36,43]. The migration we found was comparable to subsidence data from primary Exeter implants [15,38].

Particle release was observed directly after impaction. Although the bulk of the volume consisted of large titanium particles (diameter > 10 µm), the majority of the particles was smaller than 10 µm (approximately 99%). Because small titanium wear particles (< 10 µm) can lead to an inflammatory reaction and osteolysis, these particles potentially are threatening for long-term behavior of the reconstruction [20,31,41,45,47]. Not enough particles were collected during cyclic loading to be measured by the laser diffraction technique. Whether this means virtually no particles were generated during cyclic loading or that our methods to collect particles were inadequate is unclear. It is possible particles were trapped in the titanium graft layer or in the cement layer and were not flushed out during the experiment. We therefore recommend long-term animal tests to establish if any particles are generated during cyclic loading of the titanium graft and if these particles have any biologic consequences. A possible concern of the use of large amounts of titanium is systemic accumulation of titanium in the body. Elevated serum levels of titanium after (excessive) wear of titanium components have been described [10,19,23,46]. A relatively large amount of titanium in combination with the high surface area of the granules could lead to high serum levels if failure occurs. Third body wear may be another possible threat to the revision reconstruction [6,7]. Small titanium particles could be released from the graft and trapped between the head of the stem and the polyethylene cup. These particles can abrade the surface of the cup, thereby releasing osteolysis-inducing polyethylene particles. However, the access of titanium particles to the joint space can be limited by closing the graft proximally with bone cement. Additional animal tests with titanium particles should provide more insight into both phenomena.

Our data suggest titanium particles are a promising bone graft substitute from a mechanical point of view. A firm, porous layer of titanium particles can be constructed in a composite femur model. Consecutive cementing of a prosthesis in this layer results in stable reconstruction. Possible harmful titanium particles are released on impaction, but the effect of dynamic loading on particle generation is unclear. Therefore, animal tests are warranted to further investigate possible titanium particle formation and its biologic effect.

Acknowledgments

We thank Willem van de Wijdeven and Huub Peters for technical support during the experiment.

REFERENCES

1. Arroyo JS, Garvin KL, Neff JR. Arthrodesis of the knee with a modular titanium intramedullary nail. *J Bone Joint Surg Am*. 1997;79:26-35.
2. Arts JJC, Gardeniers JWM, Welten MLM, Verdonschot N, Schreurs BW, Buma P. No negative effects of none impaction grafting with bone and ceramics mixtures. *Clin Orthop Relat Res*. 2005;438:239-247.
3. Barker R, Takahashi T, Toms A, Gregson P, Kuiper JH. Reconstruction of femoral defects in revision hip surgery; risk of fracture and stem migration after impaction bone grafting. *J Bone Joint Surg Br*. 2006;88:832-836.
4. Bolder SB, Verdonschot N, Schreurs BW, Buma P. Acetabular defect reconstruction with impacted morselized bone grafts or TCP/HA particles. A study on the mechanical stability of cemented cups in an artificial acetabulum model. *Biomaterials*. 2002;23:659-666.
5. Bolder SBT, Verdonschot N, Schreurs BW, Buma P. The initial stability of cemented acetabular cups can be augmented by mixing morselized bone grafts with TCP/HA particles in bone impaction grafting. *J Arthroplasty*. 2003;18:1056-1063.
6. Bragdon CR, Jasty M, Muratoglu OK, O'Connor DO, Harris WH. Third body wear of highly cross-linked polyethylene in a hip simulator. *J Arthroplasty*. 2003;18:553-561.
7. Bragdon CR, Jasty M, Muratoglu OK, Harris WH. Third body wear testing of a highly cross-linked acetabular liner: the effect of large femoral head size in the presence of particulate poly (methyl-methacrylate) debris. *J Arthroplasty*. 2005;20:379-385.
8. Centers for Disease Control and Prevention. Hepatitis C virus transmission from an antibody-negative organ and tissue donor—United States, 2000-2002. *MMWR Morb Mortal Wkly Rep*. 2003;52:273-274, 276.
9. Conrad EU, Gretch DR, Obermeyer KR, Moogk MS, Sayers M, Wilson JJ, Strong DM. Transmission of the hepatitis-c virus by tissue transplantation. *J Bone Joint Surg Am*. 1995;77:214-224.
10. Dunstan E, Sanghrajka AP, Tilley S, Unwin P, Blunn G, Cannon SR, Briggs TWR. Metal ion levels after metal-on-metal proximal femoral replacements: a 30 year follow-up. *J Bone Joint Surg Br*. 2004;87:628-631.
11. Eldridge JDJ, Smith EJ, Hubble MJ, Whitehouse SL, Learmonth ID. Massive early subsidence following femoral impaction grafting. *J Arthroplasty*. 1997;12:535-540.
12. Fujibayashi S, Neo M, Kim HM, Tadashi K, Nakamura T. Osteoinduction of porous bioactive titanium metal. *Biomaterials*. 2004;25:443-450.
13. Galea G, Kopman D, Graham BJM. Supply and demand of bone allograft for revision hip surgery in Scotland. *J Bone Joint Surg Br*. 1998;80:595-599.
14. Gie GA, Linder L, Ling RS, Simon JP, Slooff TJ, Timperley AJ. Impacted cancellous allografts and cement for revision total hip arthroplasty. *J Bone Joint Surg Br*. 1993;75:14-21.
15. Glyn-Jones S, Alfaro-Adrian J, Murray DW, Gill HS. The influence of surgical approach on cemented stem stability: an RSA study. *Clin Orthop Relat Res*. 2006;448:87-91.
16. Goodman SB, Oh KJ, Imrie S, Hwang K, Shegog M. Revision total hip arthroplasty in juvenile chronic arthritis: 17 revisions in 11 patients followed for 4-12 years. *Acta Orthop*. 2006;77:242-250.
17. Halliday BR, English HW, Timperley AJ, Gie GA, Ling RSM. Femoral impaction grafting with cement in revision total hip replacement. Evolution of the technique and results. *J Bone Joint Surg Br*. 2003;85:809-817.

18. Holt G, Murnaghan C, Reilly J, Meek RMD. The biology of aseptic osteolysis. *Clin Orthop Relat Res*. 2007;460:240-252.

19. Jacobs JJ, Silverton C, Hallab NJ, Skipor AK, Patterson L, Black J, Galante JO. Metal release and excretion from cementless titanium alloy total knee replacements. *Clin Orthop Relat Res*. 1999;358:173-180.

20. Kumazawa R, Watari F, Takashi N, Tanimura Y, Uo M, Totsuka Y. Effects of Ti ions and particles on neutrophil function and morphology. *Biomaterials*. 2002;23:3757-3764.

21. Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. *J Bone Joint Surg Am*. 2005;87:1487-1497.

22. Landes CA, Ballon A. Five-year experience comparing resorbable to titanium miniplate osteosynthesis in cleft lip and palate orthognathic surgery. *Cleft Palate Craniofac J*. 2006;43:67-74.

23. Leopold SS, Berger RA, Patterson L, Skipor AK, Urban RM, Jacobs JJ. Serum titanium level for diagnosis of a failed, metal-backed patellar component. *J Arthroplasty*. 2000;15:938-943.

24. Malchau H, Herberts P, Eisler T, Garellick G, Söderman P. The Swedish total hip replacement register. *J Bone Joint Surg Am*. 2002;84(Suppl 2):2-20.

25. Mankin HJ, Hornicek FJ, Raskin KA. Infection in massive bone allografts. *Clin Orthop Relat Res*. 2005;432:210-216.

26. Masterson EL, Masri BA, Duncan CP. The cement mantle in the Exeter impaction allografting technique; a cause for concern. *J Arthroplasty*. 1997;12:759-764.

27. Masterson EL, Masri BA, Duncan CP, Rosenberg A, Cabanela M, Gross M. The cement mantle in femoral impaction allografting; a comparison of three systems from four centres. *J Bone Joint Surg Br*. 1997;79:908-913.

28. Morgan HD, McCallister W, Cho MS, Casnellie MT, Leopold SS. Impaction allografting for femoral component revision: clinical update. *Clin Orthop Relat Res*. 2004;420:160-168.

29. Morlock M, Schneider E, Bluhm A, Vollmer M, Bergmann G, Müller V, Honl M. Duration and frequency of every day activities in total hip patients. *J Biomech*. 2001;34:873-881.

30. Munro NA, Downing MR, Meakin JR, Lee AJ, Ashcroft GP. A hydroxyapatite graft substitute reduces subsidence in a femoral impaction grafting model. *Clin Orthop Relat Res*. 2007;455:246-252.

31. O'Connor DT, Choi MG, Kwon SY, Sung KLP. New insight into the mechanism of hip prosthesis loosening: effect of titanium debris size on osteoblast function. *J Orthop Res*. 2004;22:229-236.

32. Oonishi H, Kadoya Y, Iwaki H, Kin N. Hydroxyapatite granules interposed at bone-cement interface in total hip replacements: histological study of retrieved specimens. *J Biomed Mater Res*. 2000;53:174-180.

33. Ornstein E, Atroshi I, Franzén H, Johnsson R, Sandquist P, Sundberg M. Early complications after one hundred and forty-four consecutive hip revisions with impacted morselized allograft bone and cement. *J Bone Joint Surg Am*. 2002;84:1323-1328.

34. Papini M, Zdero R, Schemitsch EH, Zalzal P. The biomechanics of human femurs in axial and torsional loading: comparison of finite element analysis, human cadaveric femurs, and synthetic femurs. *J Biomech Eng*. 2007;129:12-19.

35. Pedersen AB, Johnsen SP, Overgaard S, Søballe K, Sørensen HT, Lucht U. Total hip arthroplasty in Denmark. Incidence of primary operations and revisions during 1996-2002 and estimated future demands. *Acta Orthop*. 2005;76:182-189.

36. Schreurs BW, Arts JJC, Verdonschot N, Buma P, Slooff TJJH, Gardeniers JWM. Femoral component revision with use of impaction bone-grafting and a cemented polished stem. *J Bone Joint Surg Am*. 2005;87:2499-2507.

37. Shahgaldi BF, Heatley FW, Dewar A, Corrin B. In vivo corrosion of cobalt-chromium and titanium wear particles. *J Bone Joint Surg Br*. 1995;77:962-966.

38. Stefánsdóttir A, Franzén H, Johnsson R, Ornstein E, Sundberg M. Movement pattern of the Exeter femoral stem: a radiostereometric analysis of 22 primary hip arthroplasties followed for 5 years. *Acta Orthop Scand*. 2004;75:408-414.

39. Sutherland AG, Raafat A, Yates P, Hutchison JD. Infection associated with the use of allograft bone from the North East Scotland Bone Bank. *J Hosp Infect*. 1997;35:215-222.

40. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. *Biomaterials*. 2005;26:6014-6023.

41. Taki N, Tatro JM, Nalepka JL, Togawa D, Goldberg VM, Rimnac CM, Greenfield EM. Polyethylene and titanium particles induce osteolysis by similar, lymphocyte-independent, mechanisms. *J Orthop Res*. 2005;23:376-383.

42. Toms AD, Barker RL, Jones RS, Kuper JH. Impaction bone-grafting in revision joint replacement surgery. *J Bone Joint Surg Am*. 2004;86:2050-2060.

43. van Biezen FC, ten Have BLEF, Verhaar JAN. Impaction bone-grafting of severely defective femora in revision total hip surgery: 21 hips followed for 41-85 months. *Acta Orthop Scand*. 2000;71:135-142.

44. van Haaren EH, Smit TH, Phipps K, Wuisman PIJM, Blunn G, Heyligers IC. Tricalcium-phosphate and hydroxyapatite bone-graft extender for use in impaction grafting revision surgery. *J Bone Joint Surg Br*. 2005;87:267-271.

45. von Knoch M, Jewison DE, Sibonga JD, Sprecher C, Morrey BF, Loer F, Berry DJ, Scully SP. The effectiveness of polyethylene versus titanium particles in inducing osteolysis. *J Orthop Res*. 2004;22:237-243.

46. von Schroeder H, Smith DC, Gross AE, Pilliar RM, Kandel RA, Chernecky R, Jugowski SJ. Titanemia from the total knee arthroplasty: a case resulting from a failed patellar component. *J Arthroplasty*. 1996;11:620-625.

47. Warashina H, Sakano S, Kitamura S, Yamauchi KI, Yamaguchi J, Ishiguro N, Hasegawa Y. Biological reaction to alumina, zirconia, titanium, and polyethylene particles implanted onto murine calvaria. *Biomaterials*. 2003;24:3655-3661.

CHAPTER FOUR

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 B Appl Biomater. 2013;101(7):1243-50.

ABSTRACT

Aims: Impaction bone grafting creates new bone stock after hip joint replacement. Utilizing a synthetic bone substitute instead of bone might increase primary stability and is not associated with graft shortage and pathogen transmission. This study compares the initial stability of a graft layer of porous titanium particles, cancellous bone particles, and a 1:2 bone-titanium mix in synthetic cemented acetabular reconstructions. Displacement was measured by radiostereometric analysis (RSA) after cyclic loading (1 Hz, maximum stress 2.5 MPa). Shear stress resistance was quantified by a lever out test of the cup. Cement penetration was quantified from cross-sections.

Findings: Titanium reconstructions showed less residual displacement (0.13 ± 0.13 mm) than pure bone particle reconstructions (0.57 ± 0.18 mm) ($p<0.01$). Titanium reconstructions were also more resistant to shear stress ($p<0.001$). The bone-titanium mix showed intermediate results. Cement penetrated deeper into the bone particle graft layers (4.8 ± 0.7) than into the titanium graft layers (3.8 ± 0.5 mm) ($p<0.02$).

Conclusions: Cemented acetabular revision reconstructions with porous titanium particles show better initial stability despite less cement penetration than bone particle reconstructions. Realistic preclinical in vivo testing should explore the hypothesis that porous titanium particles offer a safe alternative to the current gold standard of bone grafts.

INTRODUCTION

Primary and revision hip arthroplasty may be associated with large bone defects. Impaction grafting restores the original anatomy by impaction of bone particles (BoP) in the defect [1,2]. The result is relocation of the hip joint to the anatomic hip centre in a bed of new bone. This maximizes function, survival and the options for revision surgery [3-5]. BoP are contained by a mesh and entangle during impaction. The resulting macro porous graft layer shows limited cement penetration and enables ingrowth of blood vessels and host tissue which seems a prerequisite for long-term stability [6]. Despite the relatively good survival of hips reconstructed with cemented bone impaction grafting, there is room for improvement. Primary stability of the BoP layer is still a reason of concern and patients are not allowed full weight bearing during the first six to twelve weeks to enable vascularization of the graft layer. Other drawbacks of BoP also favour a fully synthetic bone substitute (need for storage equipment, shortage of donor bone, risk of pathogen transmission and religious drawbacks) [7-9]. In vitro biomechanical studies predicted the association between early migration and an increased risk of failure of a hip prosthesis by aseptic loosening in subsequent clinical studies [10-13]. Resistance of BoP reconstructions to both compressive loading and shear stress is maximized by using a cemented technique together with large particles, which can be defatted before impaction by pulse lavage [14]. Stability can be further increased by application of ceramic calcium phosphate particles (CeP) [15-17]. However, inferior handling characteristics are a serious drawback. This drawback is bypassed by mixing calcium phosphate particles with the deformable, fat containing BoP. Firstly, the mixture is more ''sticky''. Secondly, interposition of deformable bone particles between the hard calcium phosphate particles decreases pulverizing of calcium phosphate particles and the risk of fracturing the surrounding bone during impaction. Thirdly, excessive cement penetration (hindering ingrowth of host tissue and thereby graft incorporation) is prevented through obliteration of large inter-particle spaces between non-deformable calcium phosphate particles by interposition of bone particles. For these reasons it is recommended not to apply calcium phosphate particles as a full bone substitute material in impaction grafting [15-21].

In order to obtain a fully synthetic bone substitute with the favourable handling characteristics of cancellous bone, a very ductile material was chosen: particles of

porous commercially pure titanium (Hereford Metal Powder Company Ltd, Hereford, UK). Titanium particles (TiP) deform during impaction like bone grafts and create a heavily entangled and macro porous graft layer with good resistance to compressive loading [22]. TiP showed good handling characteristics and only limited cement penetration and particle generation in femoral impaction grafting [23]. In order to achieve osteo-conductive capabilities comparable to BoP and CeP, TiP should be covered with a thin (10-40 μm) bio-ceramic coating and particles should have a diameter of at least 2.8-4.0 mm [24,25]. However, the effect of particle size might not only influence bone penetration but also cement penetration and graft layer stability. The goal of this study was to evaluate the biomechanical suitability of TiP for application in acetabular cemented impaction grafting. The following hypotheses were tested:

1) A graft layer of TiP is more resistant to compressive loading and shear stress than a graft layer of BoP.
2) The stability of a graft layer of TiP is not decreased by application of a thin calcium phosphate coating.
3) Bone cement penetration depth into a graft layer of TiP or BoP is comparable.
4) Bone cement penetration depth and graft layer stability increase with an increase in TiP particle size.

MATERIALS AND METHODS

Materials

The TiP used in this study were produced during the purification of titanium through titanium tetrachloride ($TiCl_4$). This process creates a bulk of highly porous grade 1 commercially pure titanium (99.67% cp Ti). The bulk material is mechanically crumbled into smaller particles, resulting in porous titanium particles (TiP) with a wide size range. Two different size ranges were selected and tested: T34 particles (passing a sieve with 4.0 mm pores but stopped by a sieve with 2.8 mm pores) and T45 (passing a sieve with 5.0 mm pores but stopped by a sieve with 4.0 mm pores). The density of a volume of non-impacted TiP was 0.50 g/ml. Individual particles have an interconnected surface porosity of 83 ± 2% [22]. A thin (10 - 40 μm) calcium phosphate coating (carbonated apatite) was

applied to one of the titanium size groups (T34) to assess the effect of the coating on graft layer stability (Table 1). The physicochemical composition of this coating was comparable to bone and consisted of a dense layer of crystals with submicron dimensions. The coating increased the titanium particle weight by 10.5% [25]. Large morsellized cancellous bone chips were obtained by nibbling five freshly frozen (-80°C) decorticated human femoral heads with a rongeur into large particles (passing a sieve with 8.0 mm pores but stopped by a sieve with 5.6 mm pores) [22].

group	graft material	weight (g)	particle Ø (mm)	porosity (%)
B	bone	45	5.0-8.0	75 ± 4
BT34	bone, porous titanium	12.5 + 25	5.0-8.0 (bone) / 2.8-4.0 (Ti)	75 ± 4 / 83 ± 2
T34	porous titanium	30	2.8-4.0	83 ± 2
T34C	coated porous titanium	33	2.8-4.0	83 ± 2
T45	porous titanium	30	4.0-5.0	83 ± 2

Table 1 *Tested graft groups and corresponding graft material, graft weight, particle size and particle surface porosity.*

Methods

Reference value for the minimal degree of impaction of TiP

TiP can be impacted like bone particles [22]. With increasing impaction strain, the porosity decreases and mechanical strength and stiffness increase. In order to retain sufficient porosity to allow tissue ingrowth, without compromising the mechanical stability of the graft layer during postoperative in vivo loading (primary and long-term stability), a reference value was needed for the minimal amount of strain that had to be applied during impaction of TiP. A confined compression test was conducted on five non impacted TiP specimens of 3.0 grams of non coated T34 particles with cyclic loading from 0.1 – 2.5 MPa at 1 Hz during 500,000 loading cycles. A more detailed description of the confined compression test can be found in a previous published study [22]. The height of the specimen before loading was indicated by 'h_0'. During loading, the height of the specimens was recorded at minimum stress ($h_{minimum\ stress}$, height at 0.1 MPa). The loading strain ($\varepsilon_{loading}$) represents the relative deformation of the materials under dynamic loading. The loading strain was calculated as:

$$\varepsilon_{loading} = [\ln (h_0 / h_{minimal\ stress})]$$

After this test, the amount of impaction strain necessary to prevent fatigue deformation during prolonged post-operative loading was estimated to be 0.60 (95% confidence interval upper limit at the end of 500,000 loading cycles) [Figure 1]. Subsequently, five non loaded TiP specimens of 3.0 grams of non-coated T34 particles were impacted with a strain of 0.60. The height of the specimens before impaction and loading was indicated by '$h_{before\ impaction}$'. The height of the specimen after impaction and before loading was indicated by 'h_0'. The impaction strain was calculated as:

$$\varepsilon_{impaction} = [\ln (h_{before\ impaction} / h_0)].$$

Subsequently, the five impacted specimens were subjected to the same protocol of cyclic loading from 0.1 – 2.5 MPa at 1 Hz during 500,000 loading cycles. As expected, the loading strain observed in the impacted specimens was negligible (0.008 ± 0.005, equivalent to subsidence of 0.08 mm in a 10 mm thick graft layer) and did not increase between 100 and 500,000 loading cycles. From these data it was decided that the reference value for the minimal amount of strain to obtained after impaction of TiP was 0.60. This impaction strain decreases the volume of the TiP to 55% of the corresponding TiP volume before impaction.

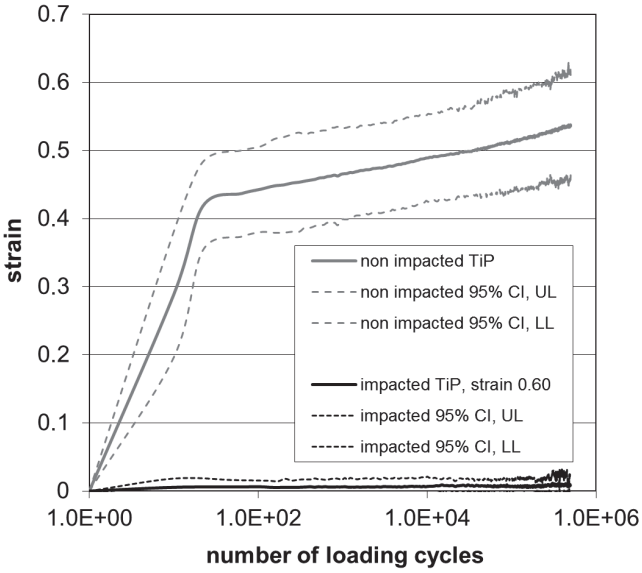


Figure 1 *Loading strain of non-impacted and impacted porous titanium particles (vertical axis) as a function of prolonged loading (x-axis, 500,000 cycles, loglinear scale).*

Acetabular reconstruction

A central cavity (Ø 60 mm), comparable to an AAOS type 2 defect, was created in cylindrical synthetic acetabular models (Sawbones Europe, Malmö, Sweden) [26]. The AAOS type 2 defect was considered large enough to assess differences in graft layer stability and is less elaborate to reconstruct than AAOS type 3 defects. Five different graft groups were tested with eight specimens per graft group (n=8). Group 1 consisted of pure cancellous BoP. Group 2 consisted of a 1:2 weight mix of BoP and non coated TiP. Groups 3, 4 and 5 consisted of pure TiP [Table 1]. The bone particle weight (45 g) per reconstruction was deducted from previous in vitro testing and evaluated by a senior hip revision surgeon (BWS). The graft layer volume of the reconstructions amounted to 32.7 ml. Assuming a density of 0.50 g/ml before impaction (non coated TiP), and an impaction strain of 0.60, this corresponds to 30 g of non-coated TiP and 33 g of coated TiP. The weight of bone particles (12.5 g) and TiP (25.0 g) used for the mix was judged to result in addition of some bone without interfering too much with the integrity of the TiP matrix. TiP were soaked in a 0.9% NaCl solution prior to application to increase cohesion between the particles. Reconstructive graft layers were shaped with a surgical hammer and semi-circular impactors (BoP), or semi-ellipsoid and finally semi-circular impactors (TiP), with diameters increasing from 20 to 46 mm to create a graft layer with a thickness of 10 mm supero-lateral and 4 mm infero-medial [Figure 2]. The polyethylene cups (Stryker Orthopaedics, Exeter Contemporary cup, outer Ø 40 mm; inner Ø 28 mm) were cemented (Simplex® Bone Cement, Stryker Orthopaedics, Limerick, Ireland) under displacement-controlled insertion using an servo-hydraulic MTS machine (MTS® Systems Corporation, Minnesota, US). All reconstructions were made by one person (LHW). After cement polymerization (24 hours at 6 ^{0} C) constructions were subjected to cyclic loading.

Cyclic loading: Five tantalum markers were attached to the cup and five to the synthetic acetabulum [Figure 3]. Secondly, the whole acetabular reconstruction was mounted in a clamp with the cup in 45 degrees inclination relative to the direction of the loading cylinder of a servo-hydraulic MTS machine. A ''time-zero'' photo (X ray 1) was made after which progressive cyclic loading at 1 Hz was started: 900 cycles from 20 – 1500 N (0.1 – 1.3 MPa) followed by 900 cycles from 20 – 3000 N (0.1 – 2.5 MPa). After a period of 300 seconds without loading (recovery period) another photo (X ray 2) was made to

record the residual displacement of the cup. Radiostereometric analysis (RSA) was used to measure residual migration and rotation by comparing the position of the cup markers and the acetabulum markers before (X ray 1) and after (X ray 2) cyclic loading. The RSA measurement error was calculated with repeated examination of measurements. The accuracy was 0.01–0.03 mm for translation and 0.01–0.08 degrees for rotation. Translations and rotations around the x-axis (medial-lateral), y-axis (cranial-caudal) and z-axis (dorsal-ventral) were calculated and combined into one cup displacement value (mm and degrees).

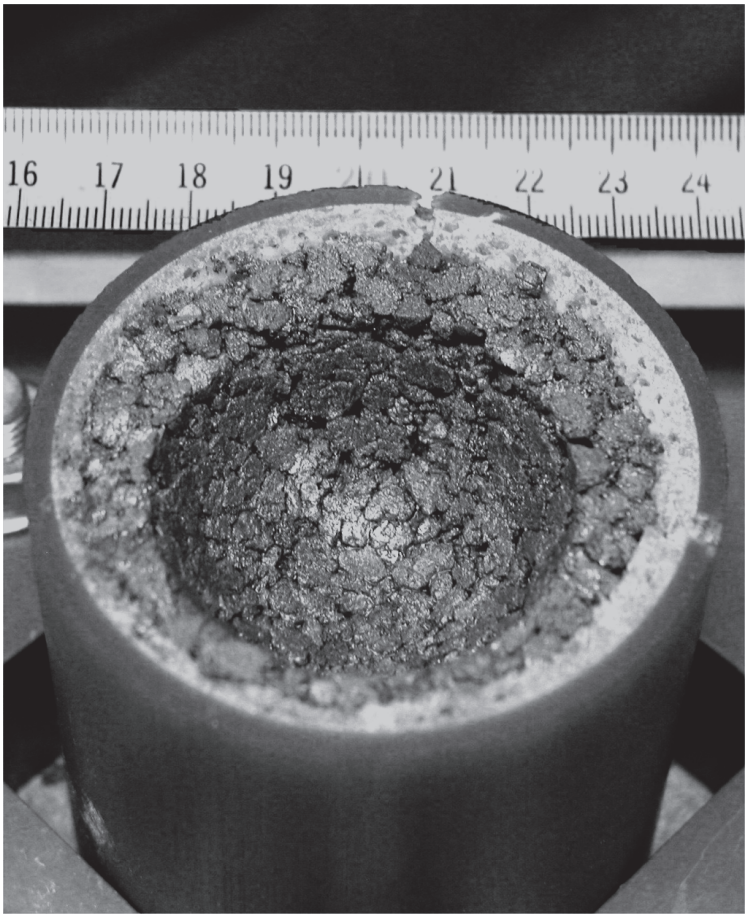


Figure 2 *A reconstructive graft layer consisting of porous titanium particles (T34). Note macro porosity and difference in supero-lateral and infero-medial graft layer thickness (10 mm vs. 4 mm). See page 199 for color figure.*

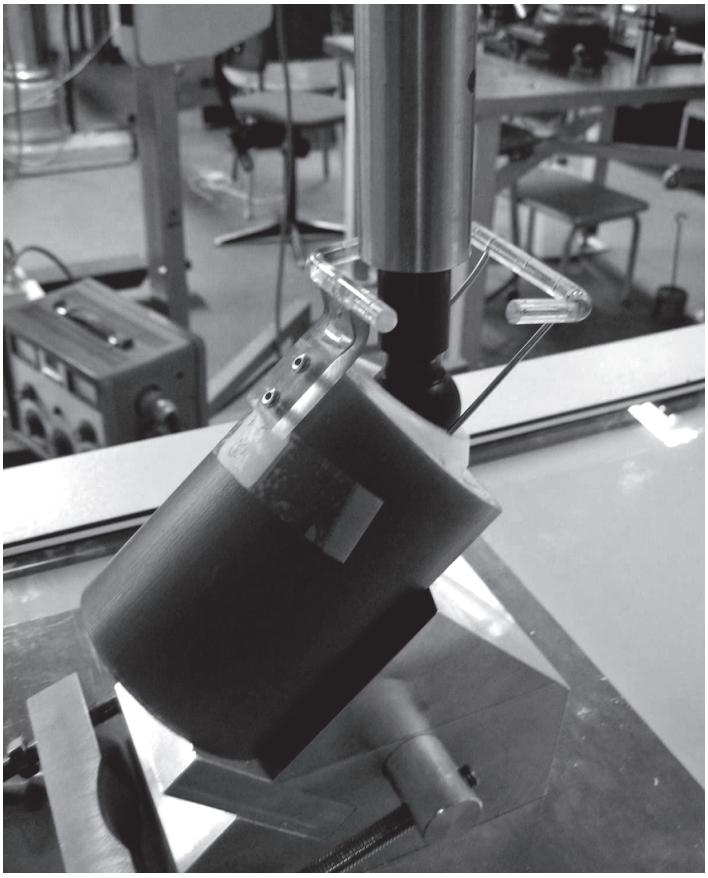


Figure 3 *Realistic in vitro cemented acetabular revision reconstruction during axial loading with RSA markers attached to both the cup and the synthetic acetabulum.*

Lever out test

After the loading test, a semicircular metal foot was fixated rigidly to polyethylene cup by pinning it to the surrounding cup with six metal rods. A metal rod was screwed on top of the semicircular foot, perpendicular to rim of the cup. A metal wire was attached to the top of the rod, perpendicular to the rod. The distance between the top of the rod and the center of rotation of the cup was 14 centimeters. The metal wire was attached to a servo-hydraulic MTS machine [Figure 4]. The metal rod was rotated supero-laterally (in the frontal plane) from a neutral position (perpendicular to the cup) until mechanical

failure of the reconstruction (''failure'') or to a maximum rotation of 30 degrees. Failure was defined as macroscopically visible detachment of the cemented cup from the reconstructed acetabulum. The force generated by the MTS machine during this movement was recorded. The maximum force was used to calculate the shear resistance of the reconstruction during supero-lateral impingement of the femoral neck against the rim of the acetabular cup:

[shear resistance (Nm)] = [maximum force (N)] x [lever distance (0.14 m)]

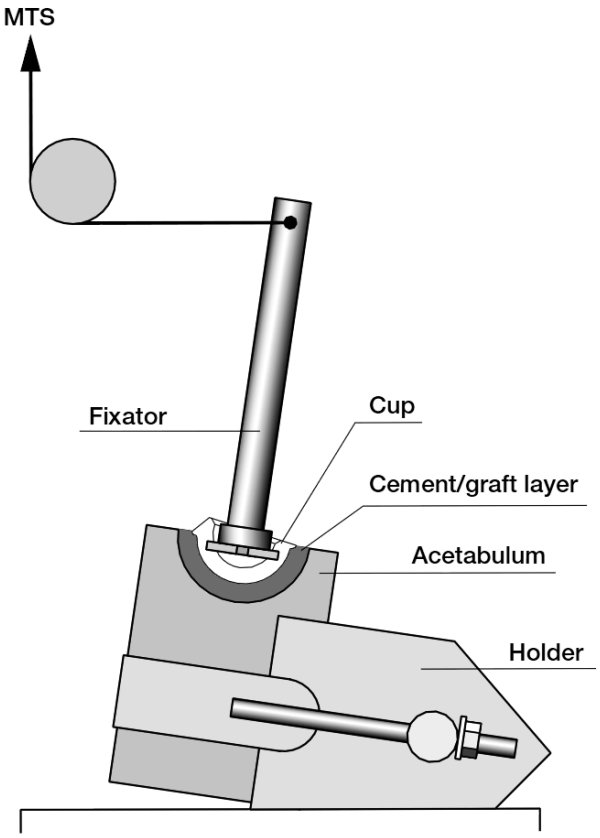


Figure 4 *The same reconstruction after loading, at the start of the lever out test with the rod fixed to the cup, and the metal pulling wire attached to the top of the rod.*

Cement penetration

All loaded reconstructions were used for quantification of cement penetration. Reconstructions were cross sectioned by a diamond coated, water cooled saw in the supero-lateral to infero-medial direction (frontal plane). After wet surface polishing finishing up to grade 800 the cross section was scanned at 1200 dpi [Figure 5]. A hemi-circle with a diameter of 46 mm was drawn to match the contour of the graft layer surface facing the cup (thin solid line). Sixteen lines were drawn from the center of the hemi-circle to the surface of the hemi-circle, corresponding to the radius, at an angular distance of 10 degrees. The sixteen lines were extended until the local interface of the cement and the titanium layer was reached. The local cement penetration was defined as the length of the dotted thin black arrow. The mean cement penetration (solid small arrow) of the reconstruction was defined as the sum of the length of the sixteen thin dotted black arrows, divided by sixteen.

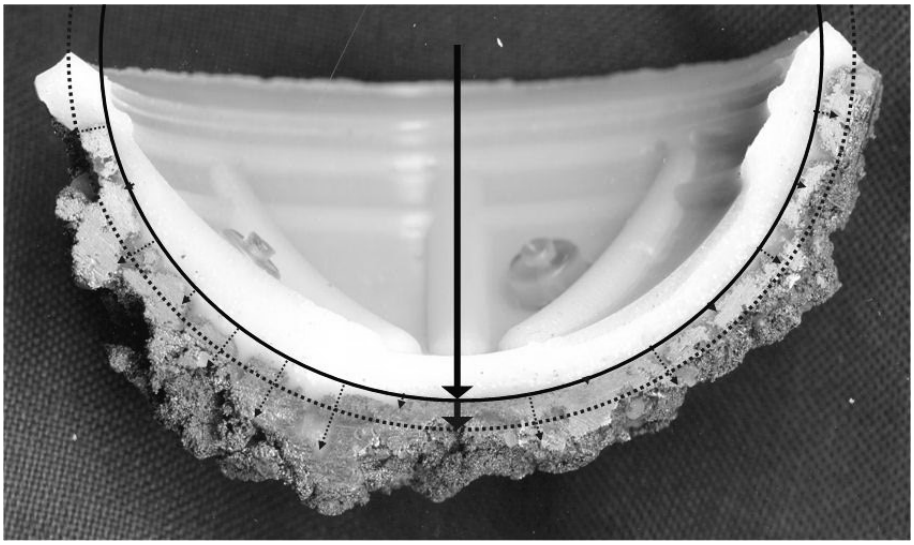


Figure 5 *Cross section of a cemented cup with titanium particles after failure during the lever out test. The solid large arrow corresponds to the radius of the largest impactor (23 mm). Local variations in cement penetration depth are indicated by the dotted small arrows. See page 200 for color figure.*

Statistics

The main measurements of interest were residual cup migration and rotation. In another study of our research group we reconstructed AAOS type 3 defects (instead of type 2 defects) [14]. These data were used to calculate a power analysis. Residual cup migration was considered 1.02 ± 0.15 mm (mean ± standard deviation). Although no precise clinically relevant migration values are available, a difference between groups in residual cup migration of 0.25 mm was considered biomechanically relevant. Power analysis with an α error of 0.05 and a power of 0.90 resulted in a group size of eight (n=8). Normality and homogeneity of variance were tested using Kolmogorov–Smirnov's and Levene's test. One way analysis of variance (ANOVA) was performed with graft group as the independent variable (SPSS 12.0). To isolate statistically significant differences, Tukey's multiple comparison procedure was used. If the assumptions of ANOVA were violated, non-parametric testing with Dunn's method was used. Results were expressed as median ± standard deviation and box plot (median, 25^{th} and 75^{th} percentile, extremes).

RESULTS

Handling of titanium particles

TiP stuck together well in the acetabular defects and deformed during impaction like BoP without fracturing of the individual particles. No excessive force was needed to shape the particles into the desired graft layer dimensions. After impaction a macro porous entangled titanium graft layer remained [Figure 2]. No differences were observed between handling characteristics of coated compared to non-coated particles. Reconstruction of the more narrow infero-medial rim (4 mm) was easier with the smaller titanium particle size (T34) compared to the larger size (T45). The bone particle graft layer was also macro porous but less entangled and less coherent. In the mixed group, BoP and bone marrow fat occluded the macro-pores between TiP. The relatively large BoP fractured during reconstruction of the narrow infero-medial rim. Impaction generated macroscopically visible debris of both bone and, to a lesser extent, titanium.

Loading

Residual cup migration (mainly subsidence) [Figure 6A] and rotation (mainly superolateral tilting) [Figure 6B] were considerably larger for bone reconstructions than for titanium groups ($p<0.001$). Migration and rotation of titanium reconstructions were not significantly changed by particle size ($p=0.72$, $p=1.00$ respectively) or application of the coating ($p=0.91$, $p=0.95$ respectively).

Lever out

All specimens with BoP or a mixture of BoP and TiP failed. Only eight out of twenty-four pure titanium reconstructions failed: three in the non-coated T34 group, three in coated T34 group, and two in the T45 group. All failed reconstructions failed within the graft layer. Non-failed titanium reconstructions showed residual plastic deformation of the cup caused by the fixation device of the metal lever-rod. Lever out forces of pure titanium reconstructions were three to four times larger than lever out forces of bone particle reconstructions ($p<0.001$) [Figure 6 C]. The titanium-bone particle mix showed intermediate results. There was no significant effect of particle size or coating on the maximum lever out torques ($p=0.92$ and $p=1.0$, respectively) of titanium reconstructions.

Cement penetration

Bone cement intrusion was found between and into individual BoP and TiP. The cement usually did not penetrate deeper than the first layer of particles facing the cement layer [Figure 6D]. This resulted in a cemented cup ''coated'' with a layer of BoP and/or TiP. Cement penetrated significantly deeper in BoP (4.8 ± 0.7) than in TiP (3.8 ± 0.5 mm, $p<0.02$). Cement penetrated slightly deeper into pure T34 particles (3.8 ± 0.7 mm) compared to T34 mixed with BoP (3.4 ± 0.4 mm). This difference was not statistically significant ($p=0.81$). Again, there was no statistically effect of particle size ($p=0.94$) or coating ($p=1.0$) [Figure 6D].

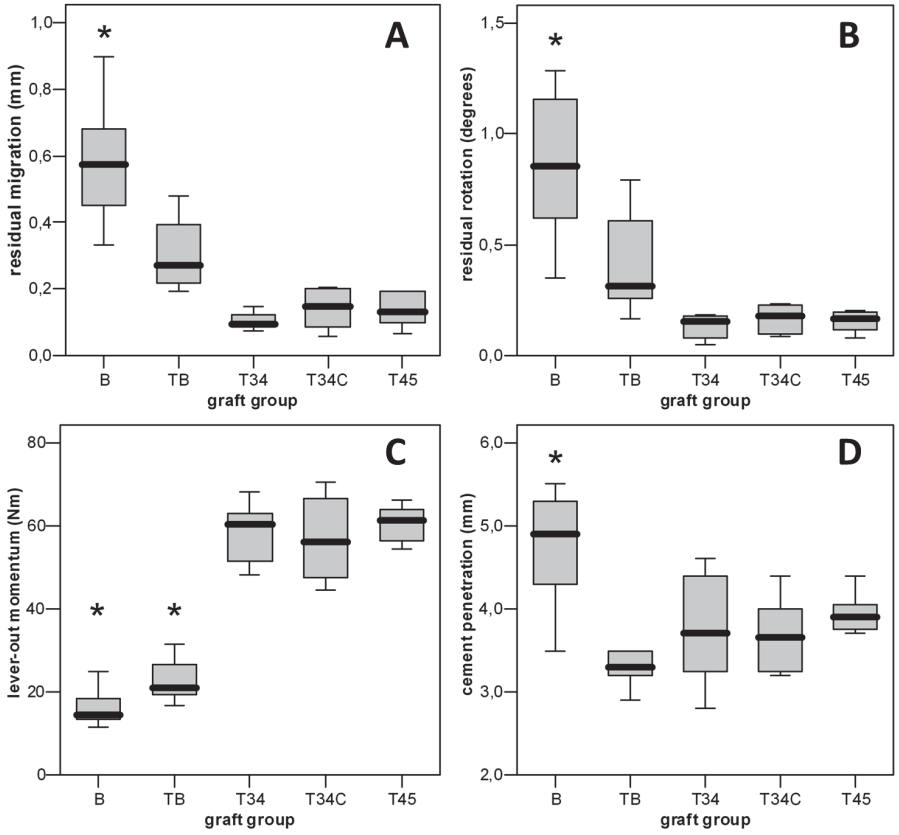


Figure 6 *Results of biomechanical analysis: A) migration; B) rotation; C) lever out momentum; D) cement penetration. Significant differences compared to all other graft groups are marked with an asterisk (*).*

DISCUSSION

The saw-bone acetabulum is a validated realistic model to compare the biomechanical stability of different graft materials in acetabular reconstructions [14-16]. Standardized defects and impaction procedures resulted in small variations within groups. In this study, primary stability was considerably increased by the application of TiP. The clinical relevance of good primary stability is well accepted. In terms of biomechanical evaluation, good primary stability means small displacement of the cup during axial loading. In order

to increase the external validity of the biomechanical evaluation, failure due to levering out of the cup during impingement of the femoral neck against the rim of the cup should be simulated as well [27,28].

Although clinical results of acetabular impaction are generally good, controversy exists about application of this technique in large defects as the results in AAOS type 3 and 4 defects are less predictable [29,30]. The available data suggest that early migration of a cup within the first *two years* of even 1.0 mm is clinically relevant and associated with long-term failure [11,12]. Arts and Bolder found a mean residual cup migration of 1.0 and 1.6 mm in reconstructed AAOS type 3 defects after only half an hour (1800 loading cycles) of loading [14,16]. Bolder observed that a 50 – 50% volume mix of bone with calcium phosphate particles, instead of 100% bone grafts, decreased residual cup migration from 0.8 to 0.6 mm (AAOS type 2 defect) and from 1.6 to 1.2 mm (AAOS type 3 defect) [16,31]. In the current study of reconstructed AAOS type 2 defects, the median residual cup migration after a similar short loading protocol decreased from 0.57 ± 18 mm to 0.13 ± 0.13 mm by application of TiP instead of bone particles. From these data, porous TiP might permit direct post-operative full weight bearing without compromising long-term reliability of the impaction grafting reconstruction.

Lever out testing showed failure of all pure bone graft reconstructions but only 8 out of 24 pure titanium reconstructions. As observed in previous studies, failure occurred within the graft layer. In line with the considerably improved resistance to axial loading, porous TiP were also four times more resistant to shear stress than large, hand nibbled BoP. Despite some room for improvement in BoP reconstructions by optimizing particle size distribution (which increases the angle of internal friction) and washing of the bone grafts (which increases the interlocking of particles) or mixing with calcium phosphate particles, the stability of the TiP reconstructions remains far superior to BoP reconstructions [14-16,32]. The explanation is the superior interlocking of the TiP within the graft layer compared to BoP and/or calcium phosphate particles. Addition of a thin bio-ceramic layer did not jeopardize this interlocking. A potential effect of particle size on graft layer stability of TiP as observed in biomechanical studies with pure BoP was not reproduced in this study [14,33]. Probably the size effect was concealed by the considerable stabilizing effect of the heavy entanglement of impacted TiP.

In general, cement did not penetrate deeper than the first layer of particles facing the cement layer. Correspondingly, smaller particle diameter (TiP) resulted in less cement penetration than large particles (BoP). Compared to a previous study of impaction grafting with TiP in synthetic femora (mean penetration 0.49 mm) the cement penetration in the acetabular graft layers was rather deep [23]. The timing of cement introduction and pressurizing in the femoral study and this acetabular study were similar. Pressures during cementation are generally somewhat higher during femoral than during acetabular application and do not explain the difference [34-37]. We calculated that the matrix of the impacted femoral graft layer (1.1 g titanium/ml) was denser than the impacted acetabular graft layer (0.9 g titanium/ml) and conclude that the higher porosity of the titanium matrix was responsible for the deeper cement penetration in this study. Two previous in vivo studies with TiP showed that a moderately impacted TiP matrix (1.1 g titanium/ml) of coated TiP with a diameter of 2.8 – 4.0 mm allows osteoconduction comparable to allograft BoP whereas a heavier impacted TiP matrix (1.0 – 2.0 g titanium/ml) resulted in occlusion of pores and blocking of ingrowing bone [24,25]. Therefore, the relatively low degree of impaction (0.9 g titanium/ml) as applied in this study is attractive from a biological point of view as cement penetration in TiP in this study is still limited compared to BoP [38].

Despite giving evidence of improved stability for the TiP reconstructions compared to application of BoP, this study also has limitations. Firstly, there was no blood or bone marrow oozing from the surrounding bone bed. This minimized the viscoelastic behaviour of the graft materials during impaction and loading. Secondly, synthetic sawbones models seem quite solid compared to the osteopenic cadaveric pelvis. This might have prevented the occurrence of a fracture during the impaction procedure. Thirdly, the impaction forces required to shape the TiP and the BoP were not quantified. This was considered redundant as standardized impaction showed equivalent or even better impactability of porous TiP compared to BoP [22]. Fourth, 1800 loading cycles represent a restricted loading protocol compared to in vivo loading. However, the largest amount of displacement seems to occur within the first thousand loading cycles [17,23]. Finally, this study focused solely on biomechanical issues, without considerations of involved biological issues. Potential biological hazards are over-impaction of the TiP (resulting in obliteration of the porous matrix and obstruction of ingrowing bone), the release of

the titanium micro particles during impaction and loading of the particles (leading to third body wear and associated osteolysis with impaired longevity of the reconstruction) and the implantation of a large surface of titanium (which might expose the patient to elevated systemic titanium concentrations).

In conclusion, TiP seems a biomechanically promising synthetic graft material for pure application in acetabular impaction grafting. TiP combine good handling characteristics with superior primary stability compared to BoP. A thin calcium phosphate coating had no negative effect on the stability of the TiP reconstructions. Cement penetration was limited compared to bone graft reconstructions. After impaction, TiP created a firmly entangled graft layer. Consequently, TiP size had no influence on cement penetration depth or graft layer stability. A realistically loaded in vivo study is indicated to address the potential biological drawbacks of application of porous TiP in impaction grafting.

Acknowledgement
Willem van de Wijdeven provided excellent technical support.

Disclosure
This study was financed by Fondel Medical B.V., Rotterdam, The Netherlands. The funder had no involvement in: the design of the study, collection, analysis and interpretation of data, writing of the manuscript and the decision to submit this manuscript for publication.

REFERENCES

1. Busch VJ, Gardeniers JW, Verdonschot N, Slooff TJ, Schreurs BW. Acetabular reconstruction with impaction bone-grafting and a cemented cup in patients younger than fifty years old: a concise follow-up, at twenty to twenty-eight years, of a previous report. J Bone Joint Surg Am. 2011;93(4):367-71.
2. Lamberton TD, Kenny PJ, Whitehouse SL, Timperley AJ, Gie GA. Femoral Impaction Grafting in Revision Total Hip Arthroplasty A Follow-Up of 540 Hips. J Arthroplasty. 2011;26(8):1154-60.
3. Flecher X, Parratte S, Brassart N, Aubaniac JM, Argenson JN. Evaluation of the hip center in total hip arthroplasty for old developmental dysplasia. J Arthroplasty. 2008;23(8):1189-96.
4. 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.
5. de Kam DC, Gardeniers JW, Hendriks JC, Veth RP, Schreurs BW. Cemented polyethylene cups in patients younger than 40 years. Clin Orthop Relat Res. 2009;467(7):1753-64.
6. van der Donk S, Buma P, Slooff TJ, Gardeniers JW, Schreurs BW. Incorporation of morselized bone grafts: a study of 24 acetabular biopsy specimens. Clin Orthop Relat Res. 2002;(396):131-41.
7. Galea G, Kopman D, Graham BJ. Supply and demand of bone allograft for revision hip surgery in Scotland. J Bone Joint Surg Br. 1998;80-B:595–9.
8. Conrad EU, Gretch DR, Obermeyer KR, et al. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg Am. 1995;77-A:214–24.
9. Cook SD, Salkeld SL, Prewett AB. Simian immunodeficiency virus (human HIV-II) transmission in allograft bone procedures. Spine. 1995;20(12):1338-42.
10. Kärrholm J, Borssén B, Löwenhielm G, Snorrason F. Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. J Bone Joint Surg Br. 1994;76(6):912-7.
11. Ornstein E, Franzén H, Johnsson R, Stefánsdóttir A, Sundberg M, Tägil 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.
12. Krismer M, Stöckl B, Fischer M, Bauer R, Mayrhofer P, Ogon M. Early migration predicts late aseptic failure of hip sockets. J Bone Joint Surg Br. 1996;78(3):422-6.
13. Hauptfleisch J, Glyn-Jones S, Beard DJ, Gill HS, Murray DW. The premature failure of the Charnley Elite-Plus stem: a confirmation of RSA predictions. J Bone Joint Surg Br. 2006;88(2):179-83.
14. Arts JJ, Verdonschot N, Buma P, Schreurs BW. Larger bone graft size and washing of bone grafts prior to impaction enhances the initial stability of cemented cups: experiments using a synthetic acetabular model. Acta Orthop. 2006;77(2):227-33.
15. Arts JJ, Schreurs BW, 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 Mech Eng [H]. 2005;219(4):257-63.
16. Bolder SB, Verdonschot N, Schreurs BW, Buma P. The initial stability of cemented acetabular cups can be augmented by mixing morsellized bone grafts with tricalciumphosphate/hydroxyapatite particles in bone impaction grafting. J Arthroplasty. 2003;18(8):1056-63.

17. van Haaren EH, Smit TH, Phipps K, Wuisman PI, Blunn G, Heyligers IC. 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(2):267-71.

18. Oonishi H, Kadoya Y, Iwaki H, Kin N. Total hip arthroplasty with a modified cementing technique using hydroxyapatite granules. J Arthroplasty. 2001;16(6):784-9.

19. Blom AW, Cunningham JL, Hughes G, Lawes TJ, Smith N, Blunn G, Learmonth ID, Goodship AE. The compatibility of ceramic bone graft substitutes as allograft extenders for use in impaction grafting of the femur. J Bone Joint Surg Br. 2005;87(3):421-5.

20. Arts JJ, Gardeniers JW, Welten ML, Verdonschot N, Schreurs BW, Buma P. No negative effects of bone impaction grafting with bone and ceramic mixtures. Clin Orthop Relat Res. 2005;438:239-47.

21. Blom AW, Wylde V, Livesey C, Whitehouse MR, Eastaugh-Waring S, Bannister GC, Learmonth ID. Impaction bone grafting of the acetabulum at hip revision using a mix of bone chips and a biphasic porous ceramic bone graft substitute. Acta Orthop. 2009;80(2):150-4.

22. Walschot LH, Schreurs BW, Buma P, Verdonschot N. Impactability and time-dependent mechanical properties of porous titanium particles for application in impaction grafting. J Biomed Mater Res B Appl Biomater. 2010;95(1):131-40.

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

24. Walschot LH, Schreurs BW, 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.

25. Walschot LH, Aquarius R, Schreurs BW, 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 B Appl Biomater. 2012;100(6):1483-9.

26. Paprosky WG, Perona PG, Lawrence JM. Acetabular defect classification and surgical reconstruction in revision arthroplasty: a 6-year follow-up evaluation. J Arthroplasty 1994;9:33–44.

27. Voigt C, Klöhn C, Bader R, von Salis-Soglio G, Scholz R. Finite element analysis of shear stresses at the implant-bone interface of an acetabular press-fit cup during impingement. Biomed Tech (Berl). 2007;52(2):208-15.

28. Marchetti E, Krantz N, Berton C, Bocquet D, Fouilleron N, Migaud H, Girard J. Component impingement in total hip arthroplasty: Frequency and risk factors. A continuous retrival analysis series of 416 cup. Orthop Traumatol Surg Res. 2011;97(2):127-33.

29. Garcia-Cimbrelo E, Cruz-Pardos A, Garcia-Rey E, Ortega-Chamarro J. The survival and fate of acetabular reconstruction with impaction grafting for large defects. Clin Orthop Relat Res. 2010;468(12):3304-13

30. van Haaren EH, Heyligers IC, Alexander FG, Wuisman PI. High rate of failure of impaction grafting in large acetabular defects. J Bone Joint Surg Br. 2007;89(3):296-300.

31. Bolder SB, Verdonschot N, Schreurs BW, 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.

32. Dunlop DG, Brewster NT, Madabhushi SP, Usmani AS, Pankaj P, Howie CR. Techniques to improve the shear strength of impacted bone graft: the effect of particle size and washing of the graft. J Bone Joint Surg Am. 2003;85-A(4):639-46.

33. Eldridge J, Hubble M, Nelson K, Smith E, Learmonth I. The effect of bone chip size on initial stability following femoral impaction grafting. J Bone Joint Surg Br. 1997;79:S3-364.

34. Wadia F, Malik MH, Leonard D, Porter ML. Cement pressurisation in the acetabulum. Int Orthop. 2006;30(4):237-42.

35. Lankester BJ, Sabri O, Gheduzzi S, Stoney JD, Miles AW, Bannister GC. In vitro pressurization of the acetabular cement mantle: the effect of a flange. J Arthroplasty. 2007;22(5):738-44.

36. Kapoor B, Datir SP, Davis B, Wynn-Jones CH, Maffulli N. Femoral cement pressurization in hip arthroplasty: a laboratory comparison of three techniques. Acta Orthop Scand. 2004;75(6):708-12.

37. Munro NA, Nicol M, Selvaraj S, Hussain SM, Finlayson DF. Femoral cement pressurization in hip arthroplasty: a comparison of 3 systems. J Arthroplasty. 2007;22(6):893-901.

38. Jones AC, Arns CH, Hutmacher DW, Milthorpe BK, Sheppard AP, Knackstedt MA. The correlation of pore morphology, interconnectivity and physical properties of 3D ceramic scaffolds with bone ingrowth. Biomaterials. 2009;30(7):1440-51.

CHAPTER FIVE

THE EFFECT OF IMPACTION AND A BIOCERAMIC COATING ON BONE INGROWTH IN POROUS TITANIUM PARTICLES

Acta Orthop 2011;82(3):372-7.

ABSTRACT

Background and purpose: Porous titanium (Ti) particles can be impacted like cancellous allograft bone particles, and may therefore be used as a bone substitute in impaction grafting. We evaluated the effect of impaction and of a thin silicated biphasic calcium phosphate coating on osteoconduction by Ti particles.

Methods: The bone conduction chamber of Aspenberg was used in goats and filled with various groups of coated or uncoated small Ti particles (diameter 1.0-1.4 mm). Impacted allograft bone particles and empty chambers were used in control groups. Fluorochromes were administered at 4, 8 and 12 weeks. Maximum bone ingrowth distance was evaluated by histomorphometric analysis.

Results: Histology of Ti particle graft cylinders showed a dense matrix with narrow inter- and intra-particle pores (<100 μm), occluding the lumen of the bone chamber. Bone ingrowth distances gradually increased with time in all groups. Maximum bone ingrowth distance was higher in originally empty chambers than those with allograft bone particles ($p=0.01$) and Ti particles ($p<0.001$). Maximum bone ingrowth in allograft bone particles was higher than in all Ti groups ($p\leq0.001$). Impaction decreased osteoconduction and the coating partially compensated for the negative effect of impaction, but these differences were not statistically significant. No osteolytic reactions were found.

Interpretation: Osteoconduction in the bone conduction chamber was reduced more by insertion of small Ti particles than by insertion of small allograft bone particles. The osteoconductive potential of porous Ti particles should be studied further with larger sized particles, which may allow bone ingrowth after impaction through larger inter-particle pores.

INTRODUCTION

Allograft bone impaction grafting restores the original bone stock (Halliday et al. 2003). Morsellized cancellous allograft bone chips remain the gold standard material, but have their limitations like limited availability, risk of pathogen transmission and religious considerations (Conrad et al. 1995, Galea et al. 1998). Calcium phosphate particles have been successfully with good long-term clinical results (Oonishi et al. 2008). However, bioceramics show inferior handling characteristics (Bolder et al 2002, van Haaren et al 2005), and accelerated resorption could compromise construct stability (Ninomiya et al 2001). Porous commercially pure titanium (Ti) particles are not resorbed by osteoclasts; thus, the stability of the graft layer is not reduced by remodelling. Long-term stability is probably dependent on ingrowth of fibrous tissue and bone. A canine model and a human retrieval have shown that non impacted, non coated small Ti particles are osteoconductive when they are applied in the femur in combination with an uncemented, vibration-based technique to insert the stem into the bed of Ti particles (Allfram et al 2007, Turner et al 2007). We intend to use larger and highly porous Ti particles with a different surgical technique: cemented impaction grafting of the acetabulum and femur. Impaction of large Ti particles creates a graft layer with good entanglement and primary stability (Aquarius et al 2009, Walschot et al 2010). However, we do not know whether impacted Ti particles allow tissue ingrowth like non impacted Ti particles: impaction poses a threat to the osteoconduction of a non-degradable material like Ti particles by obliteration of ingrowth spaces, which is even observed with a resorbable material like allograft bone (Tägil and Aspenberg 1998, Jeppsson et al 2003). Thus, we evaluated the effect of impaction on osteoconduction by small Ti particles in a bone conduction chamber (Aspenberg et al 1996) in goats. We expected that the addition of a thin sol-gel-formed silicated calcium phosphate coating would favor osteoconduction of this porous titanium graft material (Nguyen et al 2004).

ANIMALS AND METHODS

Animals

12 mature Dutch milk goats (Capra Hircus Sana) with a mean weight of 47 (range 38-59) kg were provided by the Central Animal Laboratory of the University of Nijmegen, The Netherlands. The animals were housed together in a climatologically controlled room at least 1 week before surgery (tenderfoot bottom, 18-22 ^{0} C, humidity 60 %) and provided with fresh hay, concentrate, pulp, and water.

Implanted materials

The bone conduction chamber of Aspenberg is a hollow screw (lumen: 2 mm in diameter, 7 mm high) made of commercially pure titanium with 2 ingrowth openings at the tip (Aspenberg et al 1996). It consists of 2 half-cylinders held together by a hexagonal cap with a 1 mm thick disk placed under the cap of the bone chamber to place the ingrowth openings at the level of the endostium of the goat tibia (Figure 1). 6 groups of bone conduction chambers were implanted (Table 1). A pool of mixed cancellous allograft bone particles was obtained by nibbling the freshly frozen (- 40 ^{0} C) sternums of 5 goats with a rongeur to chips of about 1 x 1 x 2 mm. Microbial culture was used to exclude contamination. After impaction specimens were stored frozen (- 40 ^{0} C) under sterile conditions and thawed before implantation. Empty bone chambers were used in a control group.

group	particulate material	graft porosity (%)
empty	no material inserted	n.a.
bone	impacted allograft cancellous bone	0.39, SD 0.09
Ti	titanium	0.78, SD 0.05
Ti_imp	titanium, impacted	0.56, SD 0.02
Ti_coat	titanium particles, with $CaPO_4$ coating	0.79, SD 0.05
Ti_imp_coat	titanium, impacted, with $CaPO_4$ coating	0.60, SD 0.05

Table *Implanted groups, corresponding graft materials and volume fractions (means, SD) from cross-sectional views of the graft cylinder.*

Porous titanium particles consist of commercially pure titanium (Hereford Metal Powder Company Ltd, Hereford, UK). The interconnected porosity of non impacted individual Ti particles was 74 (SD 4). Small Ti particles with a diameter of 1.0-1.4 mm were used. (The particles passed a sieve with pores of 1.4 mm but were stopped by a sieve with pores of 1.0 mm).

4 different groups of Ti particles were implanted: ''Ti'': not impacted, not coated; ''Ti_imp'': impacted, not coated; ''Ti_coat'': not impacted, coated; ''Ti_imp_coat'': impacted, coated. After sieving, the Ti particles were cleaned in a standardized manner to remove potential particulate and chemical pollution (procedure number PS03-016 CAM Implants BV, Leiden, The Netherlands). Sieved and cleaned Ti particles were either sterilized in an autoclave prior to (non-coated) in vivo use or processed further by application of a Bonitmatrix coating (DOT GmbH, Rostock, Germany). Bonitmatrix is a commercially available silicated biphasic calcium phosphate, which is normally available as a granular material. The Bonitmatrix substance was applied to the Ti particles as a thin coating through a sol-gel procedure at low temperatures (Teller et al 2007). The coating consists of biphasic calcium phosphate (87%; HA/TCP 60:40) and silicium dioxide (13%); it increased the weight of the Ti particles by 3.5%. The surfaces of coated Ti particles were covered with numerous ceramic granules with a mean diameter of about 5 µm. Coated Ti particles were gamma sterilizated before implantation.

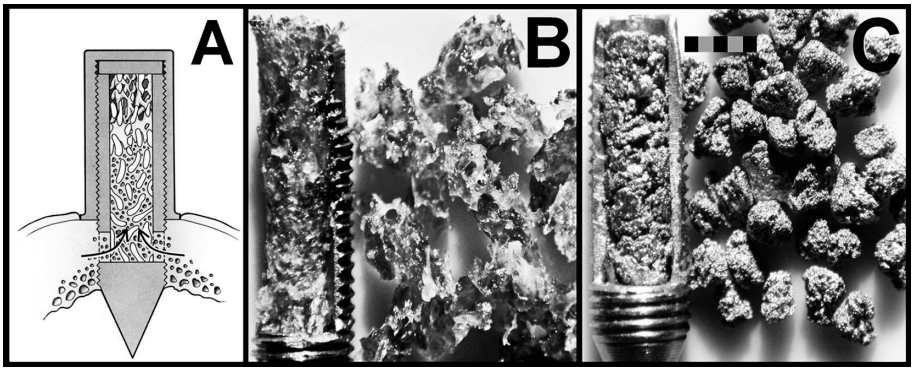


Figure 1 *A. Cross-sectional drawing of an implanted bone chamber in the proximal tibia of the goat. B. Cylinder of impacted bone particles in an opened bone chamber (left) with the corresponding amount of graft before impaction (right). C. Cylinder of impacted titanium particles (left) with the corresponding amount of graft before impaction (right). Scale bar represents 2 mm. See page 200 for color figure.*

Impaction procedure

Specimens of all graft groups were prepared by manual insertion of the corresponding graft materials into the lumen (diameter 2.0 mm) of the bone chamber. Allograft bone particles, Ti and Tci, were manually impacted with a sliding threat (diameter 1.8 mm) with a sliding weight of 9.8 g ending against a stopper at the foot of the thread. The foot of the thread was inserted in the upper lumen of the bone chamber (on top of the grafts, acting as an impactor) containing the graft particles during impaction in the rest of the lumen of the bone chamber. In order to standardize the impaction procedure, the weight was dropped 30 times from a height of 30 cm. In this way, manual impaction was mimicked as used during previous in vitro testing (Walschot et al 2010) (Figure 1). 5 non-implanted specimens were used for estimation of graft volume over total volume fraction from central longitudinal slices: mineralized bone matrix area was determined from 20 µm thin non-decalcified sections by light microscopy after Goldner staining, and cross-sectional titanium area was determined by backscatter electron imaging. Volume fractions of bone and titanium were calculated with interactive computer controlled image analysis by dividing the mineralized bone matrix area and the titanium matrix area by the whole graft cylinder area (Table).

Surgical technique

The implant position on the tibia was randomized for every bone chamber in every goat by picking folded notes from a box, with the corresponding graft group written on the (hidden) inside of the note. 12 goats were anesthetized with pentobarbital (1,200 mg) and isoflurane. A longitudinal incision over the tibia was used to expose the proximal anteromedial metaphysis. A measuring device and k-wires were used to fixate a drilling and tapping guide block which enables standardized positioning of 6 bone chambers in the tibia with a distance of at least 12 mm between chambers. After drilling and tapping of the screw holes, a round biopsy punch (6 mm in diameter) was used for excision of periosteum around the screw hole to enable direct contact between the cap of the bone chamber and the tibial cortex. Goats received 6 bone chambers in the right tibia and 6 bone chambers in the left tibia. As a result, 2 specimens of every graft group could be implanted in each goat. After the implantation procedure, the animals received analgesics and antibiotics for 3 days (subcutaneous ampicillin (15 mg/kg/48 h; 96 h), buprenorfine

(0.3 mg/12 h; 24 h), flunixine (75 mg/24 h; 72 h)). Fluorochromes were administered at 4 weeks (tetracycline (1,000 mg/24 h; 72 h)), 8 weeks (calcein green (1,250 mg/24 h; 72 h)) and 12 weeks (alizarin (1,250 mg/24 h; 72 h)). All procedures were approved by the Animal Ethics Committee of the University of Nijmegen (registration number 21034).

Evaluation

Goats were killed 12 weeks after surgery, with an overdose of pentobarbital. Bone chambers were harvested with surrounding cortex and fixed in 4% buffered formalin. After 3 days, the contents were removed from the bone chambers and fixed for another 5-7 days. Undecalcified serial slices 40 µm in thickness were made parallel to the longitudinal axis of the chamber. 3 sections were used for histological evaluation: 1 central section and 2 peripheral sections 300 µm from the central section. Maximum bone ingrowth distance was defined as the largest distance between the bottom of the bone chamber and new bone in the graft cylinder, measured parallel to the longitudinal axis of the specimen. Maximum bone ingrowth distance of a specimen was calculated as the average of maximum bone ingrowth distances in all 3 sections. Fluorescence microscopy was used to observe time dependence of bone ingrowth. After fluorescence microscopy, bone was coloured with a Goldner staining (green) or HE staining (pink) and acid phosphatase staining (osteoclasts). Bone ingrowth distances were measured with interactive computer-controlled image analysis. Backscatter electron imaging in combination with energy-dispersive spectroscopy (BEI-EDS) was used to identify and characterize micro particles.

Statistics

The study was designed to detect a difference in bone ingrowth distance of 1 mm (equivalent to the observed standard deviation in previous studies) with an α of 0.05 and a power of 0.80 in a paired study design, corrected for double implantation in every goat. 2 chambers from the same experimental group were implanted in every animal. The mean of the 2 maximum ingrowth distances in these 2 chambers was calculated, to be used for statistical analysis. The data did not show a Gaussian distribution. Repeated-measures analysis of variance on ranks (Friedman test) was used in combination with the Student-Newman-Keuls post-hoc multiple comparisons method to identify significant

differences between the graft groups (SigmaStat 3.5, Systat Software, Chicago, IL). Results for maximum bone ingrowth distance were expressed in mm as median and standard deviation (SD). The median values and tenth, twenty-fifth, seventy-fifth, and ninetieth percentiles were all used for box plot comparison.

RESULTS

Impaction procedure

Impacted allograft specimens showed a homogeneous matrix of dense and efficiently packed bone grafts. Non impacted Ti particles specimens showed inefficient packing at the periphery of the graft cylinder as a result of the relatively large diameter of the particles compared to the diameter of the inner lumen, with inter-particle pore sizes ranging from 10-500 µm. Impaction of Ti particles reduced intra-particle pore size by deformation of individual particles as well as inter-particle pore size by more efficient packing of particles. This resulted in a homogeneous and dense matrix, especially at the central part of the graft cylinder where pore sizes ranged between 10 and 100 µm. The periphery of impacted Ti particle cylinders still contained areas with larger-sized pores, due to inefficient packing (Figure 2). Ingrowth holes of the bone chamber were often obstructed by individual Ti particles, both in non impacted and in impacted Ti particle specimens.

Surgical procedure and specimen retrieval.

Implantations were technically uneventful. One goat died 5 days after operation, from an intestinal Clostridium infection complicated by sepsis. One goat suffered from a unilateral superficial wound infection, which was successfully treated by drainage and intramuscular antibiotics. Post-mortem radiographs showed unchanged implant positions and cortical thickening. During harvesting of the specimen from the lumen of the bone chamber, 4 specimens were lost from initially empty chambers and 2 specimens were lost from allograft filled chambers due to the fragility of these specimens. Ti particle cylinders showed good integrity and were not damaged during harvesting. As a result 18 ‘’empty’’ specimens, 20 ‘’bone’’ specimens and 22 specimens from every Ti particle group were available for histological evaluation.

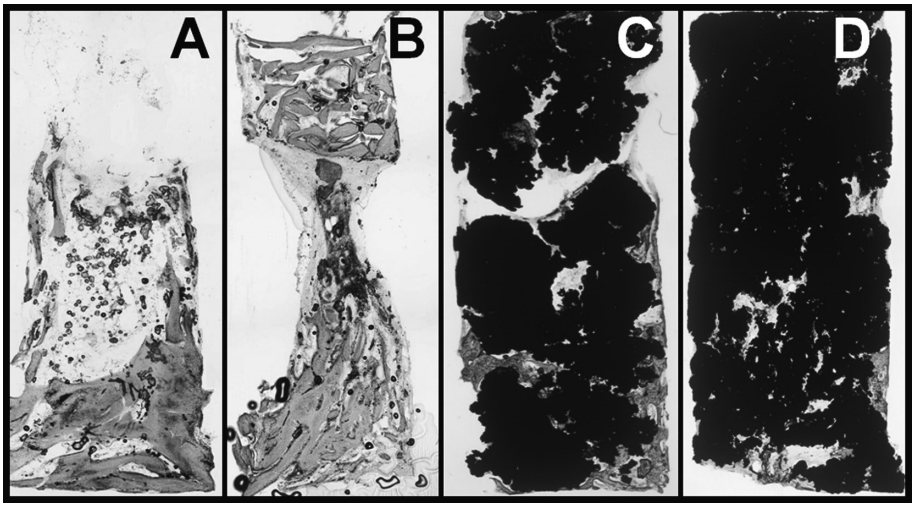


Figure 2 *Shape of the bone ingrowth front in empty chambers (A), allograft with graft remnants at the top (B), non impacted, coated titanium particles (C) and coated, impacted titanium particles (D (Goldner staining). See page 201 for color figure.*

Evaluation

Fluorochrome labelling showed only small areas of tetracycline-marked bone near the ingrowth openings or at the bottom of the chamber, which probably meant that the distant parts of bone ingrowth occurred after 4 weeks of implantation of the bone chambers. Ti particle groups and empty chambers showed broad apposition bands of calcein and narrow bands of alizarin whereas apposition bands of calcein and alizarin in allograft specimens were more evenly distributed. In empty bone chambers and bone chambers filled with Ti particles, bone first invaded along the osteoconductive wall of the chamber. This peripheral bone ingrowth pattern was more pronounced in impacted Ti particles than in non impacted Ti particles and empty chambers, and it was often not possible to determine a bone ingrowth front as in previous bone chamber studies. A clear bone ingrowth front was only observed in allografts. It was cone-shaped, in contrast to the other groups where the maximum ingrowth distance was located at the centre of the graft cylinder (Figure 2).

HE staining of some coated and non-coated Ti particle specimens suggested areas of direct contact between invading bone and the surface of the Ti particles (Figure 3). In all 4 Ti particle groups, higher-magnification views (400x) of acid phosphatase stained

specimens showed dense micro particles engulfed by macrophages at contact areas between Ti particles. Corresponding to observations by light microscopy, BEI-EDS revealed titanium micro particles of irregular shape (diameter range 1-50 µm). There were no signs of increased osteoclast numbers or macrophage-associated osteolysis (Figure 3). No traces of silicium could be found by BEI-EDS, suggesting that the Bonitmatrix coating had been fully resorbed within 12 weeks of implantation. Beside invading bone, abundant ingrowth of fibrous tissue was seen throughout the whole graft cylinder (HE staining). Fibrous armouring was also observed in impacted allograft cylinders – but to a lesser extent, which corresponds to inferior graft stability during harvesting compared to Ti particle cylinders.

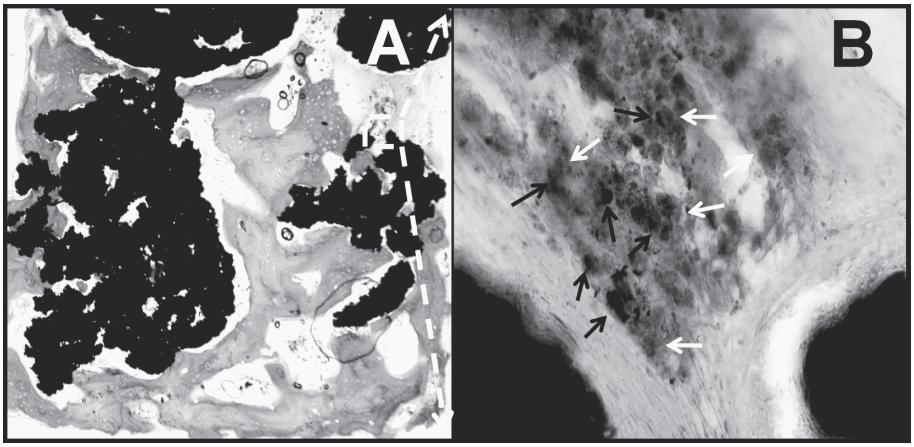


Figure 3 *A. Osteoconduction of titanium particles with bone-surface contact (HE staining). B. Acid phosphatase staining (400x) with titanium micro particles (black arrows) engulfed by macrophages (solid white arrows). See page 201 for color figure.*

Maximum bone ingrowth distances were higher in originally empty bone chambers (mean 3.2 (SD 1.9)) than in all other groups ($p \leq 0.01$). Bone ingrowth distances were intermediate in allograft bone chips (mean 1.9 (SD 1.0)) but still twice as high as in non-impacted Ti particle groups (mean 0.9 (SD 0.8); $p < 0.001$) (Figure 4). Ti particles showed lower bone ingrowth distances after impaction. Application of the silicated calcium phosphate coating partially compensated for the negative effect of impaction (mean ingrowth distance of 0.1 (SD 1.1) for non-coated, impacted Ti particles as opposed to

0.4 (SD0.9) for coated, impacted particles). However, the effects of impaction and of application of a coating did not reach statistically significant levels.

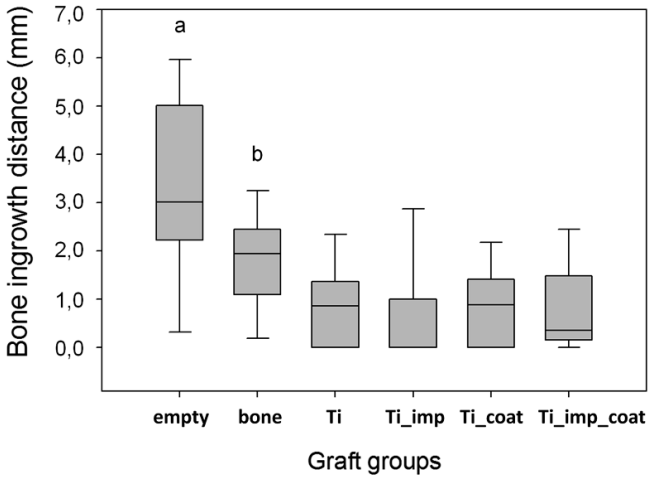


Figure 4 *Box plot of graft groups and corresponding maximum bone ingrowth distances 12 weeks after implantation. Empty bone chambers were more osteoconductive than bone chambers filled with osteoconductive bone or Ti particles (a, p≤0.01). Bone particles were more osteoconductive than Ti particles (b, p≤0.001).*

DISCUSSION

Osteoconduction by the bone chamber is sensitive to inhibition by blocking of ingrowing bone by inserted graft materials. This hypothesis is supported by impairment of bone ingrowth by impaction (Tägil and Aspenberg 1998) and the observation that OP-1 increases bone ingrowth rates in impacted allograft cylinder by accelerated graft resorption (Jeppsson et al 2003). The findings of our study are in concordance with these observations. However, some potential drawbacks of our study should be mentioned. Firstly, the bone ingrowth front appeared to be highly variable with Ti particles and was often interrupted by the presence of the Ti particles in the center of the bone chamber lumen, especially in impacted Ti particle graft cylinders. Thus, maximum bone ingrowth distance was chosen instead of the more frequently measured mean ingrowth distance

(Aspenberg et al 1996). Secondly, non impacted allografts were abandoned as a control group. Instead, empty chambers were chosen to exclude ''allograft quality'' as a potential confounder. Thirdly, 6 implants per tibia were used instead of 2 or 3. Fourthly, a Goldner stain was used instead of a hematoxylin and eosin staining, which shows more cytological detail. An additional hematoxylin and eosin stain with acid phosphatase was used in several specimens of all graft groups to identify adverse reactions such as osteolysis.

In a previous study, larger and more porous Ti particles behaved mechanically superior to bone particles (Walschot et al 2010). In our study, all Ti particle graft cylinders showed abundant fibrous armouring, which further increases graft layer strength (Tägil and Aspenberg 2001). Correspondingly, during harvesting from the implanted bone chambers, the mechanical integrity of Ti particle cylinders was superior to that of impacted allografts cylinders. Large structural allografts show only peripheral invasion by host bone (Hooten et al 1996) and require additional fixation by a cage for long-term stability (Gross and Goodman 2004). The key factor for long-term stability of bone impaction grafting reconstructions therefore seems to be the armouring by ingrowth of host bone throughout the whole allograft layer (van der Donk et al 2002). In this perspective, the small bone ingrowth distances in all Ti particle groups appear to be the main point of interest in this study.

All materials in this study were rinsed to remove chemical pollution. Bone ingrowth patterns in the allograft and originally empty control groups corresponded to previous observations (Aspenberg et al 1996). Furthermore, although the bone chamber is a non loaded model that might not be ideal for provocation of particle disease-mediated osteolysis, no signs of micro particle disease like histiocytosis, lining of bone tissue by macrophages and/ or osteoclasts, accumulation of giant cells, or toxic effects on fibroblasts (Mostardi et al 2002, Warashina et al 2003) were observed.

A mechanical rather than a biological factor seems to be responsible for the observed discrepancy between the already proven good osteoconductive properties after loose packing during intramedullary femoral application (Allfram et al 2007, Turner et al 2007) and the rather small bone ingrowth distances in more efficiently packed Ti particles in the bone chamber in this study. Osteons can grow into holes with a diameter of less than 100 µm. However, the consensus seems to be that the optimal pore diameter for ingrowth of mineralized bone lies between 100-400 µm (Itälä et al 2001). Intra-particle pores in small

Ti particles have a diameter range of about 10-100 μm, and most intra-particle pores have a diameter of <50 μm. Furthermore, small sized Ti particles also have small *inter*-particle pores which are even further compromized by some degree of impaction during insertion of the Ti particles in the bone chamber, and during subsequent deliberate impaction. As opposed to allograft bone, the matrix of porous Ti particles is not resorbable. Mechanical obstruction by narrow intra-particle pores but also narrow *inter*-particle pores could explain why the insertion of even non impacted porous Ti particles, which are made of the same material as the bone chamber itself, impaired the good intrinsic osteoconductive properties of the titanium bone chamber to a larger extent than tightly packed allografts. The impairing effect of impaction of Ti particles on the ingrowth of bone through the already small inter-particle pores could only partially be compensated for by the rather thin (5-10 μm) and fully resorbed calcium phosphate coating, which itself was not expected to exert a significant obstructive effect. The observations from a pilot study in 3 goats with cemented acetabular revision reconstructions made of considerably larger, non coated Ti particles (2.8-4.0 mm in diameter) are in agreement with our ''inter-particle pore obstruction theory'': the corresponding macro porous graft layers did indeed show local bony invasion throughout the whole graft layer with osseointegration (unpublished data).

In conclusion, Ti particles showed good fibrous armouring but inferior osteoconduction compared to allograft bone especially, especially after impaction. In this study the small Ti particles tended to obliterate the lumen of the bone chamber. Implantation of larger sized porous Ti particles in bone defects, with a clinically relevant degree of impaction, could offer an alternative to the bone conduction chamber model to evaluate bone ingrowth in a reconstructive layer of porous Ti particles. A realistic in vivo study should be conducted in order to evaluate the potentially harmful effects of titanium micro particles generated during impaction of Ti particles.

The experiment was planned by all authors. Data collection, analysis, interpretation of the data, and writing of the manuscript were done by the first author. Hereford Metal Powder Company Ltd, Hereford, UK provided the Ti particles and DOT GmbH, Rostock, Germany applied the silicated biphasic calcium phosphate coating. The study was financed by Fondel Medical B.V., Rotterdam, The Netherlands.

Léon Driessen provided excellent histologic and technical support.

Hereford Metal Powder Company Ltd., Hereford, UK provided the Ti particles and DOT GmbH, Rostock, Germany applied the silicated biphasic calcium phosphate coating. The study was financed by Fondel Medical B.V., Rotterdam, The Netherlands. The financer, the Ti particle manufacturer and the coating company had no input in the planning of the experiment, or in collection, analysis, and interpretation of the data, or writing of the manuscript.

REFERENCES

1. Allfram 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 prothetic fixation in 5 patients followed for up to 15 years. Ups J Med Sci. 2007;112(2):183-9.
2. 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 Relat Res. 2009;467(6):1538-45.
3. 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.
4. 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.
5. Conrad E U, Gretch D R, Obermeyer K R, Moogk M S, Sayers M, Wilson J J, Strong D M. et al. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg Am. 1995;77-A:214–24.
6. Galea G, Kopman D, Graham B J. Supply and demand of bone allograft for revision hip surgery in Scotland. J Bone Joint Surg Br. 1998;80-B:595–9.
7. Gross A E, Goodman S. The current role of structural grafts and cages in revision arthroplasty of the hip. Clin Orthop Relat Res. 2004;(429):193-200.
8. Halliday B R, English H W, Timperley A J, Gie G A, Ling R S. Femoral impaction grafting with cement in revision total hip replacement. Evolution of the technique and results. J Bone Joint Surg Br. 2003;85(6):809-17.
9. Hooten J P Jr, Engh C A, Heekin R D, Vinh TN. Structural bulk allografts in acetabular reconstruction. Analysis of two grafts retrieved at post-mortem. J Bone Joint Surg Br. 1996;78(2):270-5.
10. Itälä A I, Ylänen H O, Ekholm C, Karlsson K H, Aro H T. Pore diameter of more than 100 microm is not requisite for bone ingrowth in rabbits. J Biomed Mater Res. 2001;58(6):679-83.
11. Jeppsson C, Astrand J, Tagil M, Aspenberg P. A combination of bisphosphonate and BMP additives in impacted bone allografts. Acta Orthop Scand. 2003;74(4):483-9.
12. Mostardi R A, Pentello A, Kovacik M W, Askew M J. Prosthetic metals have a variable necrotic threshold in human fibroblasts: an in vitro study. J Biomed Mater Res. 2002;59(4):605-10.
13. Nguyen H Q, Deporter D A, Pilliar R M, Valiquette N, Yakubovich R. The effect of sol-gel-formed calcium phosphate coatings on bone ingrowth and osteoconductivity of porous-surfaced Ti alloy implants. Biomaterials. 2004;25(5):865-76.
14. Ninomiya J T, Struve J A, Stelloh C T, Toth J M, Crosby K E. Effects of hydroxyapatite particulate debris on the production of cytokines and proteases in human fibroblasts. J Orthop Res. 2001;19(4):621-8.
15. Oonishi H, Ohashi H, Oonishi H Jr, Kim SC. THA with hydroxyapatite granules at cement-bone interface: 15- to 20-year results. Clin Orthop Relat Res. 2008;466(2):373-9.
16. Tägil M, Aspenberg P. Impaction of cancellous bone grafts impairs osteoconduction in titanium chambers. Clin Orthop Relat Res. 1998;(352):231-8.

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

18. Teller M, Gopp U, Neumann H G, Kühn K D. Release of gentamicin from bone regenerative materials: an in vitro study. J Biomed Mater Res B Appl Biomater. 2007;81(1):23-9.

19. 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.

20. Van der Donk S, Buma P, Slooff TJ, Gardeniers JW, Schreurs BW. Incorporation of morselized bone grafts: a study of 24 acetabular biopsy specimens. Clin Orthop Relat Res. 2002;(396):131-41.

21. 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(2):267-71.

22. 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 B Appl Biomater. 2010;95(1):131-40.

23. 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.

CHAPTER SIX

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 NON-LOADED GOAT MODEL

J Biomed Mater Res B Appl Biomater 2012, 100(6):1483-9.

ABSTRACT

Aims: Impaction grafting restores bone defects in hip arthroplasty. Defects are reconstructed with bone particles (BoP). Substitute materials with adequate mechanical and biological properties are not yet available. Ceramic particles (CeP) have mechanical drawbacks as opposed to porous titanium particles (TiP). In this in vivo study, bone ingrowth and bone volume in coated and non coated TiP were compared to porous biphasic calcium phospate CeP and allograft BoP. Coatings consisted of silicated calcium phosphate and carbonated apatite. Materials were implanted in goats and impacted in cylindrical defects (diameter 8 mm) in the cancellous bone of the femur. On the basis of fluorochrome labelling and histology, bone ingrowth distance was measured at 4, 8 and 12 weeks. Cross-sectional bone area was measured at 12 weeks.

Findings: TiP created a coherent matrix of entangled particles. CeP pulverized and were non-coherent. Bone ingrowth in TiP improved significantly by the coatings to levels comparable to BoP and CeP. Cross-sectional bone area was smaller in CeP and TiP compared to BoP.

Conclusions: The osteoconductive properties of impacted TiP with a calcium phosphate coating are comparable to impacted allograft bone and impacted biphasic ceramics. A more realistic loaded in vivo study should prove that coated TiP is an attractive alternative to allograft bone.

INTRODUCTION

Osseous defects are encountered in different surgical disciplines and pose a challenge to restore the original bone stock. Bone grafting is very popular with an estimated total number of 500.000-600.000 procedures annually in the United States [1]. More specifically, osseous defects encountered in hip surgery can be restored by the impaction of cancellous bone particles (BoP) with good clinical results in the past thirty years [2,3]. Autograft bone remains the gold standard as it possesses osteoconductive, osteoinductive and osteogenetic properties [1]. Large defect sizes and donor site morbidity have lead to application of allograft bone. However, allograft bone is limited available and associated with the risk of pathogen transmission and religious restrictions [4-9]. These drawbacks could be solved by application of a fully synthetic bone substitute material. Calcium phospate ceramic particles (CeP) have been the main focus for a bone substitute material in impaction grafting [10-14]. Because of inferior handling characteristics (pulverization, fracture of the surrounding bone) and excessive cement penetration, it is advised to use CeP as a bone graft extender which still implies the application of some bone [14-16].

The handling characteristics of a graft material are of major importance for impaction grafting: the graft substitute material should be cohesive and deformable in order to shape the graft layer without fracturing the surrounding bone [15,16]. Further, the graft material should entangle during impaction which limits cement penetration and increases shear resistance [17,18]. None of the presently available resorbable bone substitute materials meets these demands. Although complete regeneration of natural tissues seems attractive, superior handling characteristics and biomechanical stability might justify the application of a biocompatible non resorbable material instead of a more biologically attractive resorbable material. From this point of view we propose deformable and highly porous titanium particles (TiP) as a potential bone substitute material for impaction grafting. TiP have favorable mechanical characteristics compared to calcium-phospate-based materials. Further, TiP is osteoconductive and provides osseointegration [19,20]. This combination of characteristics seems promising for the application of TiP as a graft substitute material in impaction grafting. In this study TiP with a diameter of 2.8-4.0 mm were used to assess the osteoconductive potential of coated and non-coated TiP. More specifically we addressed these two questions:

1. Is bone ingrowth distance comparable in TiP, BoP and CeP and time dependent within the first 12 weeks after implantation?
2. Is bone ingrowth distance in non-coated TiP comparable to bone ingrowth distance in TiP with a silicated calcium phospate coating or a carbonated apatite coating?

MATERIAL, ANIMALS AND METHODS

Implanted graft materials and physicochemical analysis of the applied bioceramic coatings

The porous titanium particles (TiP, Hereford Metal Powder Company, Hereford, UK) as used in this study were produced during the purification of titanium through titanium tetrachloride ($TiCl_4$). This process creates porous commercially pure titanium (cpTi) with a crystalline micro texture. With cross-sectional backscatter electron imaging (BEI, Jeol 6310, Tokyo, Japan) the mean porosity of TiP was calculated to be 83 ± 2% with interconnected pores. The inter-connective pore size was estimated to range between 10 and 100 µm. TiP used in this study had a diameter range of 2.8 – 4.0 mm (they passed a 4.0 mm pore sieve, but were stopped by a 2.8 mm pore sieve). After sieving, TiP were subjected to standardized cleaning to remove potential particulate and chemical pollution (procedure number PS03-016, CAM Implants BV, Leiden, The Netherlands).

Two different coatings were used: a silicated calcium-phospate coating consisting of a sol-gel coating of hydroxyl-apatite (HA) and tri-calcium-phosphate (TCP) crystals (HA:TCP 60:40) embedded in a SiO_2 layer (Bonitmatrix®, DOT GmbH, Rostock, Germany) and a coating consisting of carbonated apatite (CA, Department of Periodontology and Biomaterials, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands) [21,22]. The silicated apatite was applied as a thin coating through a sol-gel procedure at low temperatures [21]. The carbonated apatite coating was applied by submersion of the TiP in a saturated solution of calcium, phosphate, magnesium, and carbonate at 37^0 and 50^0 Celsius [22]. Physicochemical analysis of the coating was performed with X-ray diffraction (XRD, with a thin-film Philips X-ray diffractometer, using Cu Kα-radiation (PW3710, 30 kV, 40 mA)) and a scanning electron microscope (Jeol 6310) equipped with an energy disperse X-ray detector (SEM-EDS).

TiP showed a crystalline smooth surface before coating (Figure 1). The silicated calcium phosphate coating showed numerous amorphous ceramic granules with a mean diameter of about 5 μm, increased the TiP weight by 6.9% and consisted of biphasic calcium-phosphate (87 wt%) and silicium dioxide (13 wt%). The carbonated apatite coating showed a XRD pattern comparable to bone and consisted of a dense layer of crystals with a submicron size [22]. The TiP weight was increased by 10.5% and cracks were visible in the dense carbonated apatite coating as a result of the drying process. Both coatings were relatively thin (10-40 μm).

CeP (BoneSave®) is a commercially available bioceramic, constituted of 80:20 TCP:HA ratio, with a non-interconnected porosity of 50% [16]. CeP were also sieved to obtain particles with a diameter range of 2.8 – 4.0 mm. Morsellized cancellous allograft bone chips were obtained by nibbling four freshly frozen (-40°C) goat sternums with a rongeur to chips with a size comparable to TiP. BoP were preparated under sterile conditions and stored under sterile conditions at -40°C until implantation. Microbial culturing was used to exclude contamination of bone chips. Materials were subjected to gamma sterilization (50Gy) prior to implantation. Six different graft groups were implanted: BoP, CeP and four different groups of TiP (Table 1).

Implanted graft groups		
graft group	***material***	***specimen weight (g)***
B	BoP (allograft bone)	0.9
CP	CeP (βTCP/HA 80:20)	0.9
T	TiP (porous titanium)	0.55
TB	TiP/BoP	0.45/0.25
TC1	TiP/Bonitmatrix	0.59
TC2	TiP/CA	0.61

Table 1 *Implanted graft groups, corresponding materials and specimen weight.*

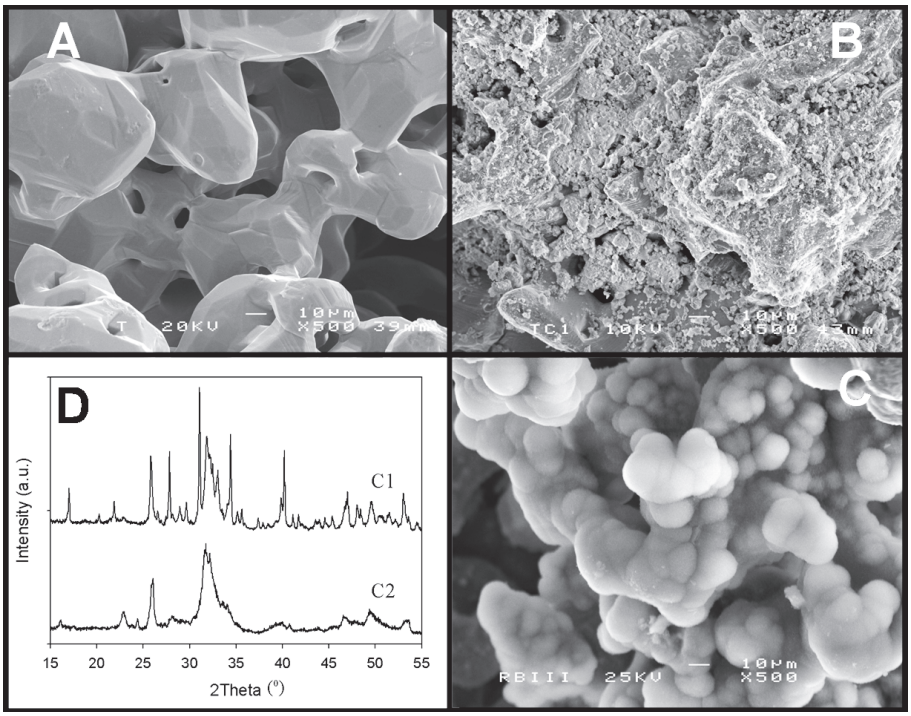


Figure 1 *Top left: TiP before coating. Top right: TiP with silicated calcium-phosphate. Bottom right: TiP with carbonated apatite (SEM, 500x). Bottom left: XRD spectra of silicated calcium-phosphate (C1) and carbonated apatite (C2).*

In vivo implantation in a non loaded goat model

Standardized impaction was used to implant the coated and non-coated TiP, allograft BoP and porous CeP in non loaded, cylindrical defects in the cancellous bone of femoral condyles of goats. Twelve Dutch milk goats (Capra Hircus Sanus) with a weight of 59 ± 10 kg (mean ± SD) were provided by the Central Animal Laboratory of the Radboud University Nijmegen, The Netherlands. Preoperatively, animals were housed group wise in a climatologically controlled room at least one week prior to surgery (tenderfoot bottom, 18-22^0 C, humidity 60 %) and provided with fresh hay, concentrate, pulp and water. Goats were anesthetized with pentobarbital (20mg/kg) and isoflurane and operated on both knees. Two longitudinal incisions (anterolateral and anteromedial) exposed the anterior part of both condyles as well as the sub-trochlear bone. Cannulated drills were used to make three cylindrical defects (sub-trochlear, medial condyle and lateral condyle) with a

diameter of 8.0 mm and a depth of 13-14 mm in every knee. Correspondingly, six defects were created in every goat, enabling implantation of one specimen of every graft group. Saline solution and low drilling speed were used to minimize heat-induced osteonecrosis. The bottom of the defect was sealed by a thin poly-tetrafluorethylene (PTFE) disc to prevent ingrowth of bone from the bottom of the defect. Graft position (right or left knee, sub-trochlear, medial or lateral condyle) was randomized preoperatively. Impaction of TiP, CeP and BoP was standardized by weighing implant materials before implantation (Table 1).

Graft cylinder volume was standardised by making a graft cylinder with a net height of 10 mm by using an impactor with a depth-marking. The applied amount of graft material, combined with the volume of the resulting graft cylinders, results in an impaction strain of 0.75-0.80 for all graft materials. This corresponds to pulverization of CeP and somewhat more vigorous impaction of BoP and TiP compared to realistic in vitro cemented acetabular reconstructions (strain 0.70 and 0.60 respectively, unpublished data). Finally, the graft cylinder was sealed by a thin polytetrafluorethylene (PTFE) disc to ensure postoperative containment. The superficial fascia and skin were closed with a resorbable braided 2.0 suture. After the implantation procedure animals received three daily intramuscular gifts of ampicillin (15 mg/kg/24h, 3 days) and also intramuscular injections of Finadyne (75 mg/24 h, 3 days) and Temgesic (0.3 mg/12 h, 2 days) for pain suppression. Postoperatively, goats were housed group wise in an outdoor farm with ample space to walk. Fluorochromes were administered during three subsequent days at 4 weeks (tetracycline 1000 mg/24 h), 8 weeks (calcein green 1250 mg/24 h) and 12 weeks (alizarin 1250 mg/24 h) after operation to monitor bone ingrowth distance in time. All procedures were approved by the Animal Ethics Committee of the University of Nijmegen, The Netherlands.

Preparation for histology and histomorphometry

Goats were killed 12 weeks after implantation with an intravenous overdose of pentobarbital (60 mg/kg). Graft cylinders were harvested with surrounding cortex and cancellous bone and fixed in 4% buffered formalin. After three days the graft cylinders were isolated by trimming the surrounding bone and additionally fixed in 4% buffered

formalin for four days and subsequently embedded in polymethylmethacrylate (PMMA). Five undecalcified slices of 30 μm thickness (at a depth of 1,3,5,7 and 9 mm respectively) could be sectioned (Leica SP1600 saw-microtome, Heidelberg, Germany) of every graft cylinder, in a plane perpendicular to the longitudinal axis of the graft cylinder. Sections 2 (3 mm depth) and 4 (7 mm depth) were not used for histological quantification but for optimizing of the Goldner staining and for analysis of coating remnants by SEM-EDS. Sections 1 (1 mm depth), 3 (5 mm depth) and 5 (9 mm depth) were used for histological quantification. First, fluorescence microscopy was used to quantify bone ingrowth distance after four weeks and eight weeks. Fluorescence microscopy could not be used to quantify the total area of bone in the section. Second, the Goldner staining was applied to colour all bone present at the moment of harvesting of the graft cylinders (12 weeks). The Goldner staining was valid for both quantification of bone ingrowth distance as well as cross-sectional bone area. All measurements were conducted by one observer (RA) according to a standardized protocol and interactive computer controlled image analysis (AnalySIS; Soft Imaging System, Munster, Germany). The observer could easily recognize the difference between BoP, CeP and TiP but was blinded for the titanium subgroups. Histological sections were digitally photographed (12.5x) and a circular region of interest with a diameter of 8.0 mm (the same diameter as the defect) was placed around the graft cylinder. The region of interest was divided into four equal segments by two crossed, perpendicular lines through the centre of the defect. Subsequently the maximum distance of bone ingrowth from the outline of the circle (periphery of the graft cylinder) towards the centre of the graft cylinder was measured in each segment, with a maximum ingrowth distance of 4.0 mm (equal to the radius of the graft cylinder). Bone ingrowth distance in a section was defined as the sum of the maximum bone ingrowth distance of all four segments, divided by four. Bone ingrowth distance of a graft cylinder was calculated as the average of bone ingrowth distance in sections 1, 3 and 5. To quantify cross-sectional bone area, the bone area (including dead allograft bone with empty lacunae in BoP graft cylinders) was quantified in the same circular region of interest as used for measurement of bone ingrowth distance. The bone area was divided by the total area of the circular region of interest and expressed as a percentage.

Statistics

Power analysis for paired comparison analysis was used to estimate the number of specimens needed to detect a minimal difference in mean bone ingrowth distance of 0.5 mm which is equal to the standard deviation observed in a previous bone chamber study (bone ingrowth distance correlation between groups 0.6, $\alpha = 0.05$, $\beta = 0.80$). One way analysis of variance (ANOVA) was performed with the factors graft group, goat and implant position to determine differences in maximum bone ingrowth distance and time dependence (SPSS 12.0). Normality and homogeneity of variance were tested using Kolmogorov–Smirnov's and Levene's test. To isolate statistically significant differences, Tukey's multiple comparison procedure was used. If the assumption of ANOVA were violated, non-parametric testing with Dunn's method was used. Results were expressed as mean ± standard deviation, median ± standard deviation and box plot (25^{th} and 75^{th} percentile, median, extremes).

RESULTS

In vivo implantation

TiP created a highly entangled matrix of compressed but still intact particles. CeP pulverized and created a dense matrix of many small and non-connected particles. BoP appeared to fracture during impaction and showed some entanglement. Two CeP graft cylinders failed during creation of the defect due to minor penetration of the subchondral plate in one goat and an extra-articular fracture of the cortex of the lateral condyle in another goat. These defects were reconstructed but left empty. All goats were allowed full weight bearing directly after operation. One goat suffered from an postoperative infectious arthritis which was clinically successfully treated by incision and drainage and administration of ampicillin. The infected knee showed secondary erosive osteoarthritis. However, no histological difference was seen compared to graft specimens in the contralateral non-infected knee. During retrieval of the graft cylinders, the two empty defects did not show any tendency for bone ingrowth.

Histomorphometry

Goldner staining resulted in green coloring of bone. BoP specimens showed complete covering of original graft remnants (empty lacunae) by newly formed bone (filled lacunae). The dense matrix of pulverized CeP was diffusely connected by ingrowing bone. Ingrowth distances were smaller in non-coated TiP than in coated TiP ($p < 0.001$) and other groups ($p < 0.001$) at all time points. Bone ingrowth distance was time dependent and increased significantly in five out of six graft groups between four and twelve weeks of implantation (Table 2, Figure 2). TiP cylinders showed that *inter*–particle pores (ranging from 50 to 200 µm) were larger than *intra*–particle pores (ranging from 10 to 100 µm). Bone ingrowth in non–coated TiP specimens was restricted to the relative large inter–particle pores at the periphery of the graft cylinder (Figure 3). However, application of a coating resulted in presence of bone throughout the whole graft cylinder, both in *inter*–particle pores as well as small *intra*–particle pores. Application of a bioceramic coating increased bone ingrowth distance in TiP to a larger extent than mixing with BoP.

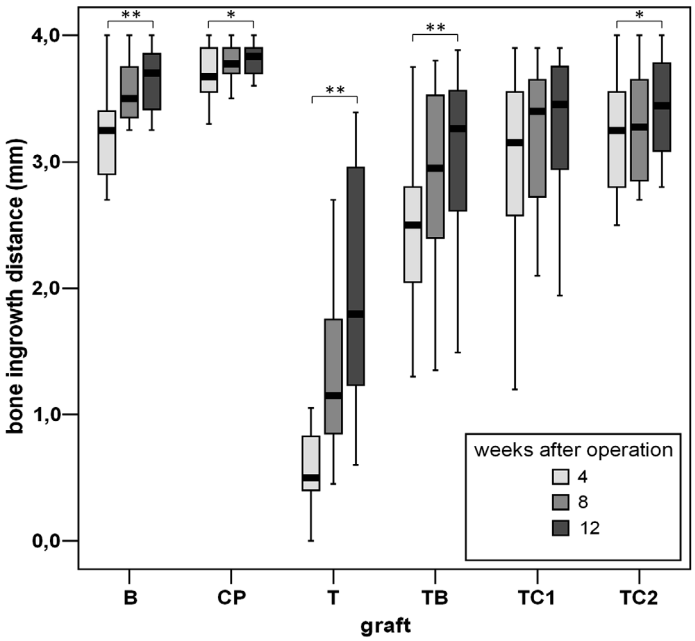


Figure 2 *Bone ingrowth distance after 4, 8 and 12 weeks. Significant time dependence within a graft group between all time points was marked by one ($p<0.05$) or two ($p<0.001$) asterisks (*).*

	Time dependence of bone ingrowth		
graft group	*distance at 4 weeks*	*distance at 12 weeks*	*p-value*
B	3.2 ± 0.4	3.7 ± 0.3	<0.001
CP	3.7 ± 0.3	3.8 ± 0.1	0.02
T	0.6 ± 0.5	2.0 ± 1.0	<0.001
TB	2.3 ± 1.0	3.0 ± 0.8	<0.001
TC1	2.9 ± 0.9	3.2 ± 0.8	0.21
TC2	3.1 ± 0.7	3.3 ± 0.6	0.02

Table 2 *Graft groups and mean bone ingrowth distances (mm) after 4 and 12 weeks.*

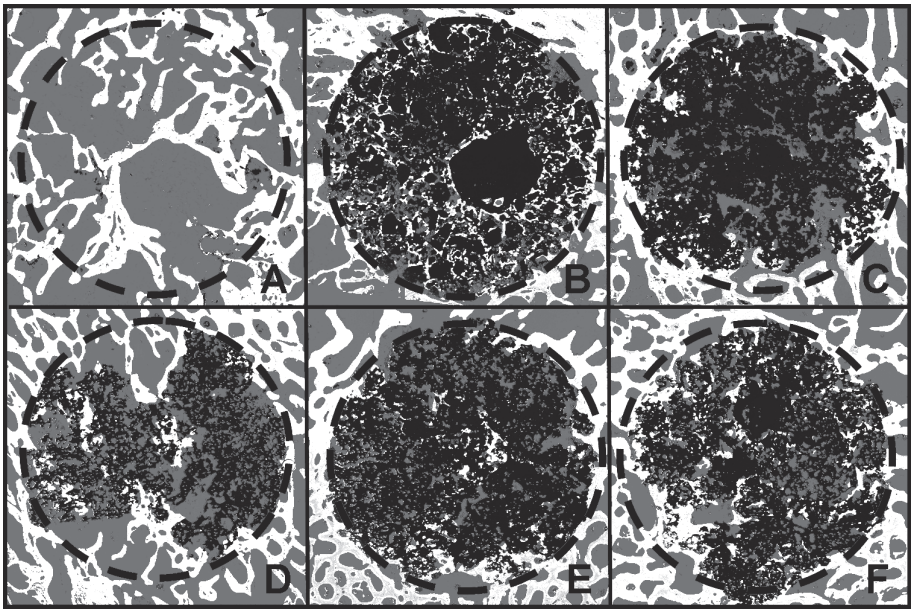


Figure 3 *Representative cross-sections 12 weeks after implantation, showing presence of bone (white) in the center of all graft groups except non-coated TiP (A-F: B, CP, T, TB, TC1, TC2). The dotted circle outlines the original defect. See page 202 for color figure.*

Cross-sectional bone area percentage was largest in BoP specimens. Bone area percentage was significantly higher in BoP than in non-coated and coated TiP ($p<0.05$). CeP specimens also showed significantly more bone area than non-coated TiP ($p<0.05$). However, the difference between CeP and coated TiP did not reach significant levels. The increase in bone ingrowth depth by addition of a bioceramic coating was reflected by a somewhat larger cross-sectional bone area percentage in coated TiP (median 7.6 ± 3.1 and 8.2 ± 4.9)

compared to non-coated TiP (5.3 ± 3.7, Figure 4). There was a trend towards more, and less variable bone ingrowth in TiP with the carbonated apatite coating (TC2) compared to TiP coated with silicated calcium-phosphate (TC1) ($p>0.05$).

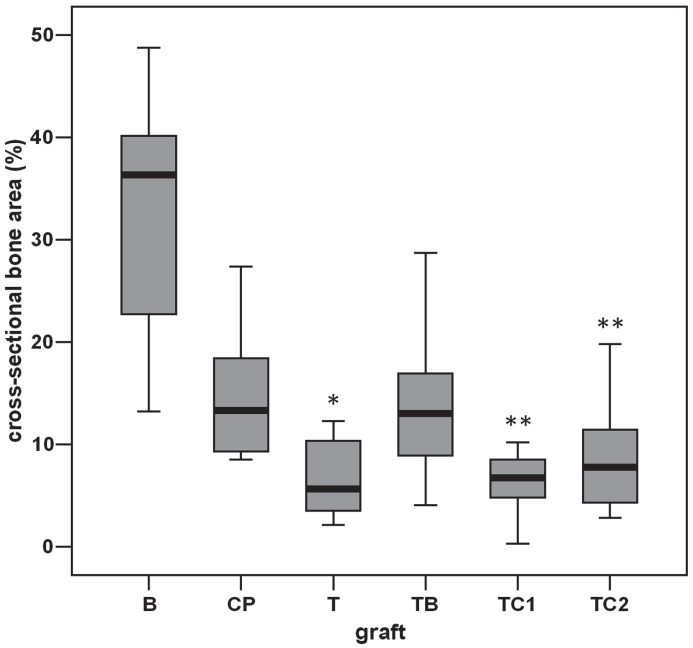


Figure 4 *Cross-sectional bone area percentage after 12 weeks. Significant differences between non-coated TiP and other groups (B, CP and TB) were marked with one asterisk ($p<0.05$). Significant differences between TC1 and TC2 and other groups (B) were marked with two asterisks ($p<0.05$).*

SEM-EDS could not trace any coating remnants. Observations of titanium micro-debris particles in cross-sections, generated during the impaction procedure, were only sparse even at high power views. No adverse histological reactions like collections of macrophages and giant cells or demarcation of newly formed bone by osteoclasts could be observed. BEI showed direct contact between the surface of TiP and newly formed bone (osseointegration, Figure 5).

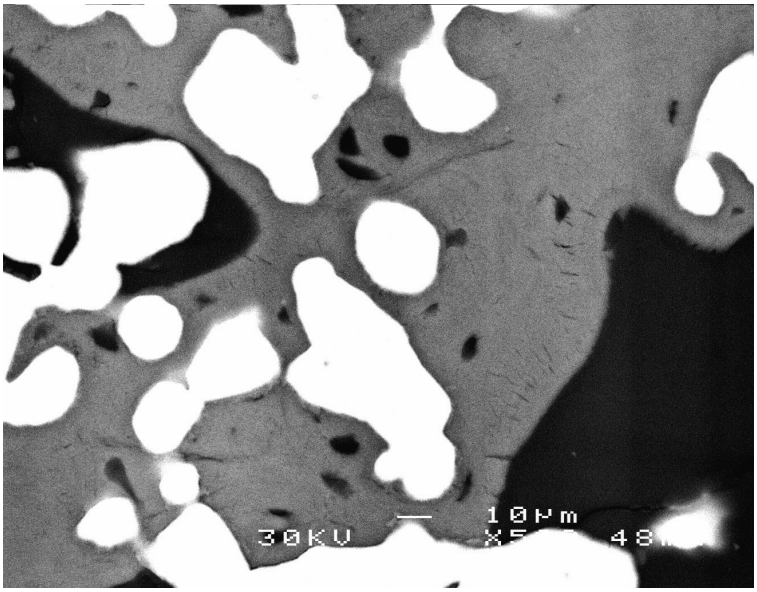


Figure 5 *Osseointegration with direct contact between invading bone (gray, with black osteocyte lacunae) and the surface of the titanium particle (white; BEI 500x, 30kV). The small white line indicates 10 µm.*

DISCUSSION

Products of porous metal are widely used in orthopedic surgery as primary and revision implants and more recently as augments for bony defects [23-25]. TiP possess mechanical characteristics favorable for impaction grafting like good handling, impactability and resistance to compressive loading [18]. In addition, from this study it seems that impacted TiP with a thin bioceramic coating possess osteoconductive properties comparable to allograft bone. Potential drawbacks have to be considered: Firstly, it is not clear whether the used defect is critically sized. Theoretically, the inserted graft materials might be osteo-*obstructive* instead of osteo-*conductive*, decelerated bony bridging of the defect compared to leaving the defect open. However, for reasons of external validity (bone defects in hip surgery have to be filled in order to guarantee primary stability of the arthroplasty during initial loading) it was decided not to use an empty control group and to include CeP as an extra control group. Secondly, graft materials were tested under

non loaded conditions. Soft callus formation is known to be stimulated by loading-induced micromotions [26]. Non loaded conditions could have concealed an effect of graft elasticity on osteoconduction and bone formation within the graft matrix. However, calcium-phosphate deposition was observed in the middle of elastic (BoP), intermediate (TiP) and stiff (CeP) graft materials as early as 4 weeks after implantation. Thirdly, the release and effect of titanium micro-debris was not addressed in this study. Although two in vitro studies with TiP showed rather small amounts of impaction debris and no signs of fretting corrosion, the absence of loading could have minimized a potential cytotoxic or osteolytic effect of the generated micro particles [18,27,33]. Finally, graft particles were rather small compared to allograft particles used in impaction grafting. Bone ingrowth is pore size dependent, an issue which has to be addressed especially when a non resorbable material like TiP is used. The optimum pore size for bone ingrowth is considered to range from 100 – 400 µm [28]. Smaller particles result in smaller spaces between particles (inter-particle pores) which could interfere with vascularization of ingrowing bone especially in the non resorbable impacted titanium particles. In a previous non loaded in vivo study, graft cylinders made of 1.0 – 1.4 mm TiP showed pore sizes ranging between 10 and 100 µm. Because of the small pore sizes, ingrowing bone was blocked [29]. The larger 2.8 – 4.0 mm TiP as used in this study, created graft cylinder pores ranging from 10 to 200 µm, enabling bone ingrowth in both inter-particle pores (coated and non-coated TiP) as well as in smaller intra-particle pores (coated TiP), suggesting that pores were not obliterated by the applied coatings.

The clinical success of allograft reconstructions is imputed to the extensive incorporation process [30]. The question remains if the observed amount of bony armouring is sufficient for long-term stability of the TiP reconstructions. The observed cross-sectional bone area fractions appear rather small compared to BoP specimens. However, a major part of the bone area in BoP specimens consisted of mechanically inferior allograft remnants as the bone volume fraction of non impacted trabecular bone of osteoporotic goats is already as high as 18% [31]. The required amount of new bone ingrowth in TiP might be quite small, if necessary at all, as the acetabular subchondral trabecular human bone volume fraction itself usually does not exceed 20% [32]. Moreover, the non resorbable TiP matrix provides superior mechanical stability compared to CeP and BoP and is further reinforced by fibrous armouring [18,29,33,34]. CeP showed cross-sectional bone area fractions

comparable to coated TiP. Despite this low bone volume fraction CeP perform clinically well in the biomechanically challenging environment of acetabular reconstructions [13,14].

In conclusion, TiP with a diameter of 2.8 – 4.0 mm and a thin bioceramic coating seem a promising bone graft substitute material for impaction grafting. Ingrowth of bone was time dependent. The large inter–particle pores (≥ 100 μm) seem a prerequisite for bony armouring throughout the whole reconstructive layer of TiP. The effect of penetrating bone cement, cyclic loading, exposure to synovial fluid and generated titanium micro particles should be studied under more realistic conditions to prove that TiP with a bioceramic coating is a safe alternative to bone particles in impaction grafting.

Acknowledgement

Léon Driessen provided excellent histomorphometry support. The funder (Fondel Medical B.V.) had no involvement in: the design of the study, collection, analysis and interpretation of data, writing of the manuscript and the decision to submit this manuscript for publication.

REFERENCES

1. Goldberg VM, Stevenson S. Natural history of autografts and allografts. Clin Orthop Relat Res. 1987;(225):7-16.
2. Busch VJ, Gardeniers JW, Verdonschot N, Slooff TJ, Schreurs BW. Acetabular reconstruction with impaction bone-grafting and a cemented cup in patients younger than fifty years old: a concise follow-up, at twenty to twenty-eight years, of a previous report. J Bone Joint Surg [Am]. 2011;93(4):367-71.
3. Ornstein E, Linder L, Ranstam J, Lewold S, Eisler T, Torper M. Femoral impaction bone grafting with the Exeter stem - the Swedish experience: survivorship analysis of 1305 revisions performed between 1989 and 2002. J Bone Joint Surg [Br]. 2009;91(4):441-6.
4. Rajan GP, Fornaro J, Trentz O, Zellweger R. Cancellous allograft versus autologous bone grafting for repair of comminuted distal radius fractures: a prospective, randomized trial. J Trauma. 2006;60(6):1322-9.
5. Jones AL, Bucholz RW, Bosse MJ, Mirza SK, Lyon TR, Webb LX, Pollak AN, Golden JD, Valentin-Opran A; BMP-2 Evaluation in Surgery for Tibial Trauma-Allgraft (BESTT-ALL) Study Group. Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. J Bone Joint Surg [Am]. 2006;88(7):1431-41.
6. Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg [Am]. 2002;84-A(5):716-20.
7. Galea G, Kopman D, Graham BJ. Supply and demand of bone allograft for revision hip surgery in Scotland. J Bone Joint Surg [Br] 1998;80-B:595–9.
8. Conrad EU, Gretch DR, Obermeyer KR, Moogk MS, Sayers M, Wilson JJ, Strong DM l. Transmission of the hepatitis-C virus by tissue transplantation. J Bone Joint Surg [Am] 1995;77-A:214–24.
9. Cook SD, Salkeld SL, Prewett AB. Simian immunodeficiency virus (human HIV-II) transmission in allograft bone procedures. Spine. 1995 15;20(12):1338-42.
10. Stein JM, Fickl S, Yekta SS, Hoischen U, Ocklenburg C, Smeets R. Clinical evaluation of a biphasic calcium composite grafting material in the treatment of human periodontal intrabony defects: a 12-month randomized controlled clinical trial. J Periodontol. 2009;80(11):1774-82.
11. Burstein FD, Williams JK, Hudgins R, Boydston W, Reisner A, Stevenson K, Cohen S. Hydroxyapatite cement in craniofacial reconstruction: experience in 150 patients. Plast Reconstr Surg. 2006;118(2):484-9.
12. Kanayama M, Hashimoto T, Shigenobu K, Yamane S, Bauer TW, Togawa D. A prospective randomized study of posterolateral lumbar fusion using osteogenic protein-1 (OP-1) versus local autograft with ceramic bone substitute: emphasis of surgical exploration and histologic assessment. Spine. 2006;31(10):1067-74.
13. Oonishi H, Ohashi H, Oonishi H Jr, Kim SC. THA with hydroxyapatite granules at cement-bone interface: 15- to 20-year results. Clin Orthop Relat Res. 2008;466(2):373-9.
14. Blom AW, Wylde V, Livesey C, Whitehouse MR, Eastaugh-Waring S, Bannister GC, Learmonth ID. Impaction bone grafting of the acetabulum at hip revision using a mix of bone chips and a biphasic porous ceramic bone graft substitute. Acta Orthop. 2009;80(2):150-4.

15. van Haaren EH, Smit TH, Phipps K, Wuisman PI, Blunn G, Heyligers IC. 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(2):267-71.

16. Arts JJ, Schreurs BW, 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 Mech Eng H. 2005;219(4):257-63.

17. Oakley J, Kuiper JH. Factors affecting the cohesion of impaction bone graft. J Bone Joint Surg [Br]. 2006;88(6):828-31.

18. Walschot LH, Schreurs BW, Buma P, Verdonschot N. Impactability and time-dependent mechanical properties of porous titanium particles for application in impaction grafting. J Biomed Mater Res B Appl Biomater. 2010;95(1):131-40.

19. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. Biomaterials. 2005;26(30):6014-23.

20. Wohlfahrt JC, Monjo M, Rønold HJ, Aass AM, Ellingsen JE, Lyngstadaas SP. Porous titanium granules promote bone healing and growth in rabbit tibia peri-implant osseous defects. Clin Oral Implants Res. 2010;21(2):165-73.

21. Teller M, Gopp U, Neumann HG, Kühn KD. Release of gentamicin from bone regenerative materials: an in vitro study. J Biomed Mater Res B Appl Biomater. 2007;81(1):23-9.

22. Leeuwenburgh S, Layrolle P, Barrère F, de Bruijn J, Schoonman J, van Blitterswijk CA, de Groot K. Osteoclastic resorption of biomimetic calcium phosphate coatings in vitro. J Biomed Mater Res. 2001;56(2):208-15.

23. Gustke KA. The Natural-Knee system: 25 years of successful results. Am J Orthop (Belle Mead NJ). 2010;39(6 Suppl):5-8.

24. Patil N, Lee K, Goodman SB. Porous tantalum in hip and knee reconstructive surgery. J Biomed Mater Res B Appl Biomater. 2009;89(1):242-51.

25. Kawanabe K, Ise K, Goto K, Akiyama H, Nakamura T, Kaneuji A, Sugimori T,Matsumoto T. A new cementless total hip arthroplasty with bioactive titanium porous-coating by alkaline and heat treatment: average 4.8-year results. J Biomed Mater Res B Appl Biomater. 2009;90(1):476-81.

26. Jagodzinski M, Krettek C. Effect of mechanical stability on fracture healing--an update. Injury. 2007 Mar;38 Suppl 1:S3-10.

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

28. Itälä AI, Ylänen HO, Ekholm C, Karlsson KH, Aro HT. Pore diameter of more than 100 micrometer is not a prerequisite for bone ingrowth in rabbits. Biomed Mater Res. 2001;58(6):679-83.

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

30. Ullmark G, Sörensen J, Långström B, Nilsson O. Bone regeneration 6 years after impaction bone grafting: a PET analysis. Acta Orthop. 2007 Apr;78(2):201-5.

31. Li Z, Peng S, Pan H, Tang B, Lam RW, Lu WW. Microarchitecture and nanomechanical properties of trabecular bone after strontium administration in osteoporotic goats. Biol Trace Elem Res. 2012 Jan;145(1):39-46.

32. Dalstra M, Huiskes R, Odgaard A, van Erning L. Mechanical and textural properties of pelvic trabecular bone. J Biomech. 1993 Apr-May;26(4-5):523-35.

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

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

CHAPTER SEVEN

POROUS TITANIUM PARTICLES FOR ACETABULAR RECONSTRUCTION IN HIP ARTHROPLASTY SHOW EXTENSIVE BONY ARMOURING AFTER FIFTEEN WEEKS. A LOADED IN VIVO STUDY IN TEN GOATS

Submitted to Acta Orthopaedica

ABSTRACT

Background and purpose: The bone impaction grafting technique (BIG) restores defects in hip arthroplasty by impaction of bone particles. Porous titanium particles (TiP) are deformable like bone particles and offer better primary stability. The following questions were addressed: are impacted TiP osteoconductive under loaded conditions, do released micro-particles accelerate wear and does the application of TiP cause elevated systemic titanium levels?

Animals and methods: An AAOS type 3 defect was created in the right acetabulum of ten goat, 63 (SD 6) kg, and reconstructed with calcium phosphate coated TiP and a cemented polyethylene cup. A stem with a cobalt chrome head was cemented in the femur. Goats were killed after fifteen weeks. Blood samples were taken pre- and postoperatively.

Results: The TiP graft layer measured 5.6 (SD 0.8) mm with a bone ingrowth distance of 2.8 (SD 0.8) mm. Cement penetrated 1.0 (SD 0.5) mm into the TiP. One reconstruction showed minimal cement penetration (0.3 mm) and failed at the cement-TiP interface. No signs of accelerated wear, metallic particle debris or osteolysis were observed. Systemic titanium concentrations increased on a log-linear scale from 0.5 (0.3-1.1) to 0.9 (0.5-2.8) ppb ($p=0.01$).

Interpretation: Adequate cement pressurizing is advocated for impaction grafting with TiP. After implantation, calcium phosphate coated TiP were osteoconductive under loaded condition 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 prove that application in humans is safe in the long-term.

INTRODUCTION

One out of twenty people will suffer from arthritis of the hip and the associated considerable decrease in physical, social and mental function [1-3]. Although primary THA does not fully restore the level of pain and physical functioning, patient satisfaction is high (>90%) and durable (>15 years) [4-6]. In 2005 as many as 650.000 primary THA's and 100.000 revision THA's were performed in the US and Europe. This number will continue to increase in the coming decades due to demographic factors [3, 7-9]. It is a trend to operate patients at a younger age. Due to the secondary nature of arthritis in younger patients (rheumatoid arthritis, osteonecrosis, congenital hip dysplasia) it is often this patient who presents with an acetabular defect at primary THA [10]. A total hip prosthesis has a limited life span and failure usually occurs at the acetabular side [11]. As a result, the young (< 50 years) patient is also at risk for one or multiple revision procedures with the associated progressive bone loss [12, 13]. A major challenge in both primary and revision THA is dealing with these bone deficiencies.

Different surgical techniques can be used to deal with acetabular defects. Of these techniques, only bone impaction grafting (BIG) has proven to combine all desired features such as adequate positioning of the joint, good long-term survival and bone stock regeneration [14-17]. However, BIG becomes more challenging in large defects and high failure rates have been reported in AAOS type III and IV defects [18-20]. In clinical studies of BIG, radiographic follow-up at five years showed only loosening in cups with high rates of initial migration and rotation as measured by radiostereometric analysis [21, 22]. More extensive impaction grafting reconstructions have thicker graft layers and biomechanical studies have shown that failure usually occurs within the graft layer [23]. Therefore, the key to success with the impaction grafting technique is to maintain sufficient primary stability throughout the revitalizing phase. This revitalization process might take several years depending on the thickness of the reconstructive layer and is characterized by revascularization with partial graft resorption and subsequent temporarily mechanical weakening [21, 24].

Calcium phosphate particles increase the primary stability of the graft layer [25]. However, due to inferior handling characteristics and the risk of third body wear these calcium phosphate particles are not applied as a full bone substitute material in impaction grafting

[26]. In the search for a more ductile material, porous titanium particles (TiP) were tested. TiP are deformable like cancellous bone particles and result in acetabular reconstructions with a superior mechanical stability [27, 28]. Furthermore, impacted TiP with a coating of calcium phosphate are osteoconductive like impacted cancellous bone particles under non loaded conditions [29]. However, the application of TiP has not been tested under realistic in-vivo loading conditions. The goal of this study was to evaluate the application of calcium phosphate coated TiP in a realistic loaded goat defect model. More specifically, the following hypotheses were tested:

1) A impacted layer of coated and impacted TiP is stable and osteoconductive under loaded conditions.
2) The amount of titanium micro particles released by TiP will not cause adverse tissue reactions or increased wear.
3) Implantation of a large effective surface of titanium will increase systemic titanium concentrations.

MATERIALS, ANIMALS AND METHODS

Materials

The porous titanium particles (TiP, Hereford Metal Powder Company Ltd, Hereford, UK) used in this study were produced during the purification of titanium (Ti) through titanium tetrachloride (TiCl4). This process creates a bulk of highly porous grade 1 commercially pure titanium (99.67% cp Ti). The bulk material is mechanically crumbled into smaller particles, resulting in porous titanium particles (TiP) with a wide size range. One size range was used: particles passed a sieve with 4.0 mm pores but were stopped by a sieve with 2.8 mm pores. Sieved TiP were cleaned to remove potential particulate and chemical pollution (procedure number PS03-016, CAM Implants BV, Leiden, The Netherlands). The density of a volume of non impacted TiP measured 0.50 g/ml. Individual particles have an interconnected surface porosity of 83 (SD 2) % [29]. The calcium phosphate coating is constituted of carbonated apatite and is relatively thin (10-40 μm) (Figure 1). The coating was applied by submersion of TiP in a saturated calcium phosphate containing solution at 37 °C and 50 °C and subsequently dried at 50 °C according to a previously

published protocol [29, 30]. Coated TiP were sterilized by 50 kGray gamma radiation prior to implantation (Isotron Nederland® B.V., Ede, The Netherlands).

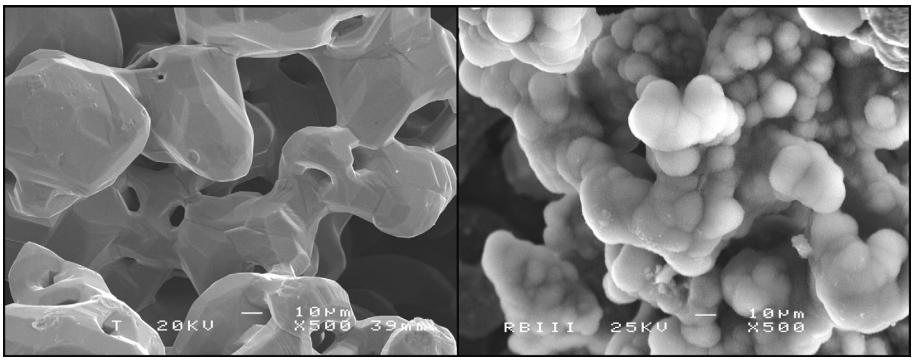


Figure 1 *Left: TiP before coating. Right: TiP coated with carbonated apatite (SEM, 500x).*

Animals and surgical technique

Ten mature Dutch milk goats (Capra Hircus Sanus, Central Animal Laboratory of the University of Nijmegen, The Netherlands) with a weight of 63 (SD 6) kg were operated. Preoperative management consisted of antibiotic prophylaxis (intramuscular injection, Baytril 0.2 mL/kg; Bayer, Division Animal Health, Mijdrecht, The Netherlands) and intramuscular administration of both buprenorphine hydrochloride (Temgesic 5 mg/kg; Renckitt Benkiser Healthcare, Hull, UK) and a non-steroidal anti-inflammatory drug, fluxin meglumine (Finadyne 1 mg/kg; Schering-Plough Animal Health, Brussels, Belgium). Goats were anesthetized with pentobarbital (30 mg/kg; Ceva Sante Animale, Maassluis, The Netherlands) and isoflurane (2.5% isoflurane on an oxygen/nitro-oxygen mixture). 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 thereafter the femoral head was dislocated. Next, a femoral neck osteotomy was performed. The acetabulum was reamed up to a diameter of 32 mm. Subsequently, the superolateral and anteromedial rims were removed with a high speed power drill to simulate a large combined cavitary and segmental defect (AAOS type III). The acetabulum was reconstructed with a metal mesh

(X-Change, Stryker Orthopaedics, Newbury, UK) which was secured to the outer side of the pelvic bone with four AO bone screws (diameter 3.5 mm; length 10-20 mm, 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 5-10 g of TiP with X-change comparable revision instruments (hemi-elliptical and hemi-spherical impactors varying in diameter from 14-32 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 minutes after mixing the powder with the monomer and thereafter pressurized for 2 min. An Exeter sheep polyethylene cup (inner diameter, 22.2 mm; outer diameter, 29 mm, Stryker Orthopaedics) was inserted 6 minutes after mixing of the cement (Figure 2D). Frequently, the anti-dislocation rim of the cup had to be downsized manually to accommodate the defect. After setting of the acetabular cement, the femoral shaft was opened and prepared with broaches. The femoral canal was lavaged and bone cement was injected retrograde 3.5 min after mixing of the cement. A polished V40 Exeter sheep stem (made of cobalt chrome (CoCr) with a corresponding V40 22.2 mm CoCr femoral head, Stryker, Benoist Girard, France) was inserted 5 minutes after mixing the bone cement. After setting of the cement, the hip was reduced. Next, the abductors and endorotators were reattached and the soft tissues were closed in layers. Subsequently an X-ray was made of the hip region (Figure 2E). Postoperatively, the animals were placed in a supporting hammock for 2 weeks which allowed full weight bearing. After the implantation procedure animals received three intramuscular gifts of ampicillin (Albipen LA 15 mg/kg; Intervet, Boxmeer, The Netherlands) and also intramuscular injections of Finadyne (75 mg/24 h, 3 days) and Temgesic (0.3 mg/12 h, 2 days) for pain suppression. After 2 weeks, the goats were housed in an outdoor farm with ample space to walk around. Fluorochromes were administered during three subsequent days at four weeks (tetracycline 15 mg/kg/24h), eight weeks (calcein green 20 mg/kg/24h) and fifteen weeks (alizarin 20 mg/kg/24 h) after operation to monitor bone ingrowth distance in time. All procedures were approved by the Animal Ethics Committee of the University of Nijmegen, The Netherlands (DEC number 2006-025, project number 21044, 18 December 2006).

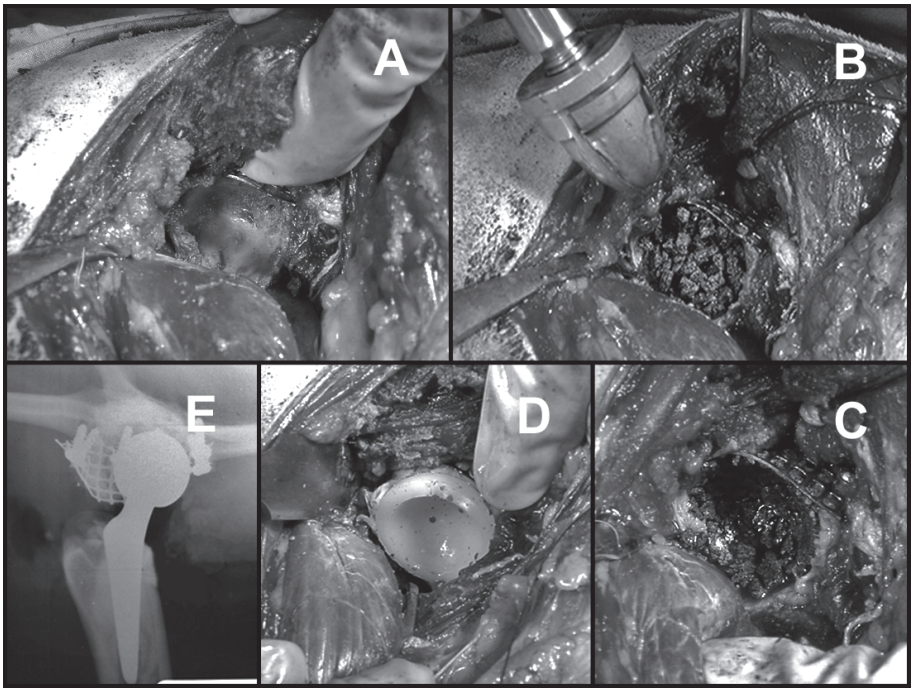


Figure 2 *A-E: Reconstruction of an acetabular AAOS type III defect (A) with TiP (B), impaction grafting (C) and a cemented downsized Exeter hip prosthesis (D and E). See page 203 for color figure.*

Histomorphometry

Goats were killed with an intravenous overdose of pentobarbital (60 mg/kg). Standard roentgen photographs were taken from the implant sites to verify the implant position and to exclude fractures and dislocations. Both the femur and the reconstructed acetabulum were harvested, cleaned from soft tissue and fixed in a 4% buffered formaldehyde solution at 4°C for at least 10 days. After removal of the cup the reconstructed acetabular defects were sectioned with a diamond saw into radially orientated slices (Figure 3). The slices were left non-decalcified and were embedded in PMMA. Serial sections of 30 µm (Leica SP1600 saw-microtome, Heidelberg, Germany) were alternatively left unstained for fluorescence microscopy or stained with haematoxylin and eosin (HE). Serial non stained 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 TiP layer at the TiP-cement interface was drawn. The center of the corresponding circle resembled the center of the final semi-circular impactor. Twelve lines were drawn from the center of the 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. Thickness of the fibrous tissue interface between cement and the TiP layer.
2. Penetration depth of cement from the TiP-cement interface into the TiP layer.
3. Penetration depth of ingrowing bone from the TiP-bone interface into the TiP layer.
4. Thickness of the TiP layer.
5. Thickness of the fibrous tissue interface between the TiP layer and the surrounding host bone.

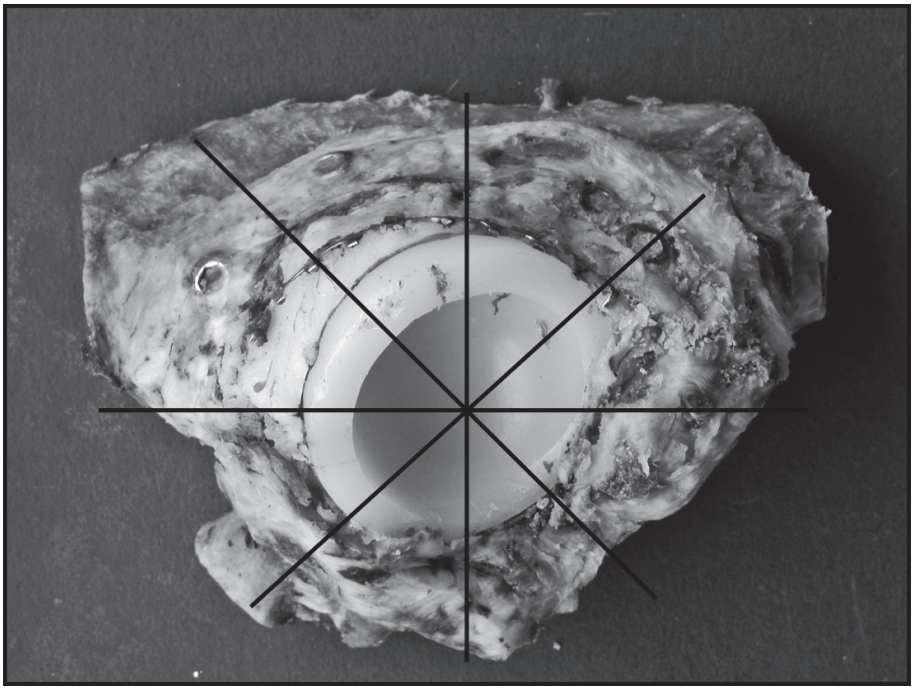


Figure 3 *Retrieved acetabular reconstruction fifteen weeks after implantation. The black lines indicate the orientation and location of the sections used for histomorphometry. Superolaterally, the rim of the metal mesh is visible as well as the heads of the screws used for initial fixation of the mesh.* *See page 204 for color figure.*

Wear analysis

Pre-operatively the surface of four new polyethylene cups and four new CoCr heads was inspected at different magnifications with scanning electron microscopy (Jeol 6310) equipped with an energy disperse 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 pre-operatively. For comparison of damage, the articular surface of a retrieved cup from a previous goat study was used (acetabular impaction grafting with a mix of bone particles and calcium phosphate particles) [26].

Systemic titanium analysis

Whole peripheral blood was collected at standardized moments (just before surgery and postoperatively at 1, 7, 28, 56 and 105 days) in metal-free vacutainers (BD Vacutainer® Trace Element, BD Diagnostic Systems, Belgium) and frozen (-40 0 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 (Co, control) and titanium (Ti) content using an inductively coupled plasma mass dynamic reaction cell spectrometer (ICP-MS) with an AS-93 auto sampler (Perkin Elmer, Massachusetts, USA). 500 µl of whole blood was mixed with 500 µl HNO3 0.7 %, 500 µl HNO3 70 % and incubated overnight (12 hours – 14 hours) at 80 °C in a polypropylene tube of 15 ml (Greiner Bio-One GmbH, Frickenhausen, Germany). The next day 8.4 ml HNO3 0.7 % and 100 µl rhodium internal standard (Chem-lab CL01.11811.0100, Zedelgem, Belgium) were added. Ultra-pure HNO3 was obtained after sub-boiling distillation in the laboratory of HNO3 70 % (Across 424000025, Waltham, USA). Ultra-pure water was obtained from an Elgastat maxima (Elga Ltd., Bucks, UK). Titanium (Ti 49.9448) was measured with ammonia (0.85 ml/min) as a reaction gas to minimize interferences, 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 (Chem-lab CL01.13774.0100, Zedelgem, Belgium) at three concentrations for each element. The detection limits in the sample matrix were 0.08 µg/l (cobalt) and 0.3 µg/l (titanium).

Statistics

Univariate linear regression was performed with the factors goat (random) and time (covariate) as independent variables to determine time dependence of systemic titanium and cobalt concentrations (SPSS 20.0). A log transformation was applied for the whole blood titanium and cobalt concentrations to meet the criteria of univariate analysis. Results were expressed as mean (standard deviation), median (range), box plot (median, 25th and 75th percentile, extremes) and ppb (parts per billion = µg/l).

RESULTS

Animals, surgical technique and retrieval of acetabular reconstructions

Surgical procedures were uneventful. TiP showed excellent handling properties and deformability comparable to cancellous bone particles. Between 5.0-8.0 g TiP were implanted in every goat. Macroscopically no release of titanium micro particles was observed during or following impaction of the TiP. Goats started loading of the operated leg within a few hours after surgery. One goat suffered from a femoral fracture three days postoperatively and was euthanized. Therefore, nine goats were available for analysis. None of them had wound infections and all goats showed a symmetrical gait. Post-mortem X rays and retrieval of the acetabular reconstructions showed that one goat had a dislocated femoral head without other abnormalities of the acetabular reconstruction (goat nine). Further, one reconstruction turned out to have failed at the cement-TiP interface: the cemented cup had detached with a part of the cement layer from the still intact TiP graft layer (goat three). None of the reconstructions showed signs of mechanical breakdown of the TiP graft layer. Additionally, there were no macroscopic signs of adverse histologic events like infection, synovitis or metallosis.

Histomorphometry

Cement penetration was clearly visible at the non-decalcified cross-sections of the acetabular cups, 0.87 (0.35-1.87) mm (Figure 4A, Figure 5). Bone ingrowth distance averaged 2.79 (0.76) mm and the whole graft layer thickness averaged 5.56 (0.85) mm (Figure 5). In the HE stained sections the pink stained bone was seen in all specimens

penetrating into the larger gaps between the individual TiP (Figure 4B). The reconstruction that failed at the cement-TiP interface showed only very shallow cement penetration, 0.35 mm, with direct contact between cement and the TiP over only 43% of the interface (HE stained sections). The eight other reconstructions showed cement penetration at least twice as deep, 0.87 (0.79-1.87) mm, with more direct contact between the cement and the TiP, 78 (SD 28%). If present, the fibrous interface between cement and TiP was rather thin, 0.13 (0.02-0.77) mm. The fibrous tissue interface, measured over the whole cement-TiP interface distance, averaged 0.05 (SD 0.05) mm (Figure 5).

At the interface between the host bone and the TiP layer there was close and direct contact between bone and the TiP in all reconstructions (Figure 4C). This new bone was stained by fluororochromes (Figure 4D). Also the smaller pores within the individual TiP showed the presence of new bone (Figure 4E). Higher magnifications of fluorescence microscopy and HE straining 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 TiP 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 TiP. Consequently, if present, the fibrous tissue interface was rather thick, 0.87 (SD 0.70) mm (Figures 4F-H). However, measured over the whole distance of the TiP-bone interface, the fibrous interface averaged only 0.01 (0.00-0.19) mm (Figure 5). Analysis of the TiP graft layer showed that inter–particle pores (ranging from 50-1000 µm) were larger than intra–particle pores (ranging from 10-100 µm). Analysis of the different fluorochromes labels showed penetration of new bone throughout the whole depth of the graft layer, both through the larger inter-particle pores as well as through the smaller sized intra-particle pores, as early as four weeks after implantation (tetracycline, yellow). A gradual increase in the width and depth of the ingrowing bone was observed between four to fifteen weeks after implantation. At many locations the bone, invading the TiP 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).

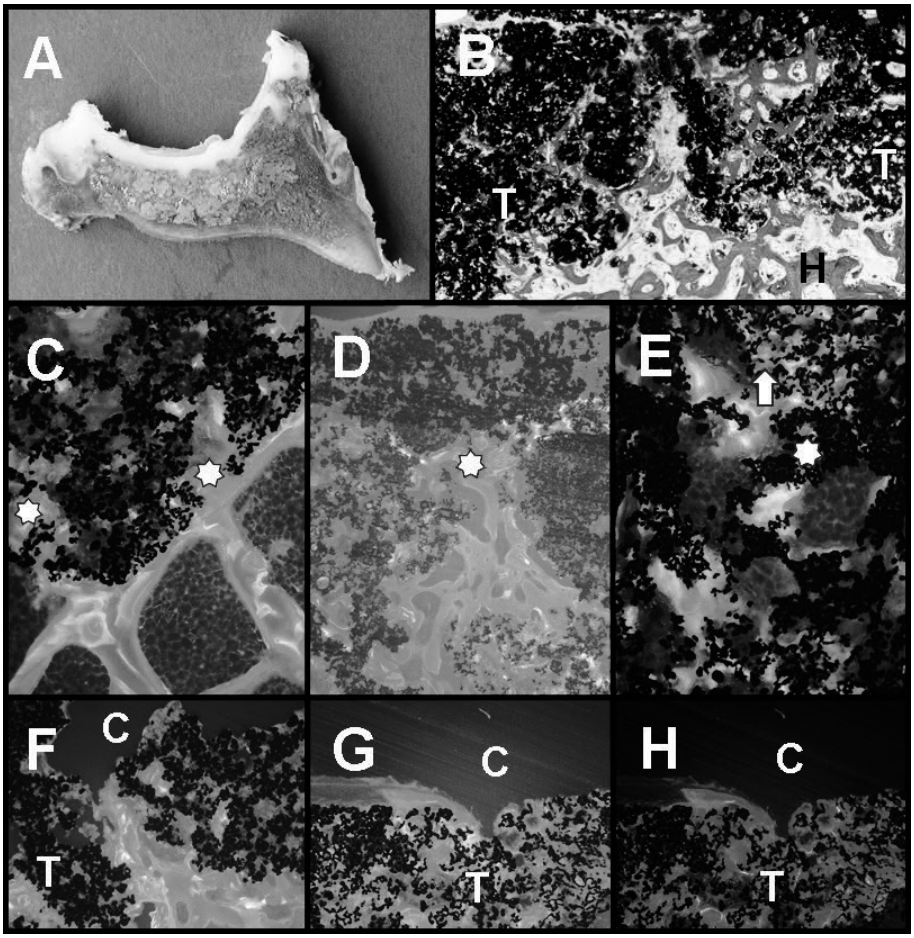


Figure 4 *A. Cross-section of reconstructed acetabulum showing the host bone (H), TiP layer (T), and cement (C). B. HE stained detail of the host bone (H, gray) TiP (T, black) interface. C. Detail of host bone-TiP layer with calcein fluoresce. Asterisks indicate new bone. D. New bone formation in gap between two TiP particles (asterisks). E Calcein fluorescence indicating new bone formation in larger (asterisks) and smaller pores (white arrow) in TiP particle. F. TiP(T)-cement (C) interface. G and H Same section and location but with different filter sets showing calcein and tetracycline fluorescence (G) and alizarin fluorescence (H). Notice thin soft tissue interface between TiP (T) and Cement layer (C). See page 205 for color figure.*

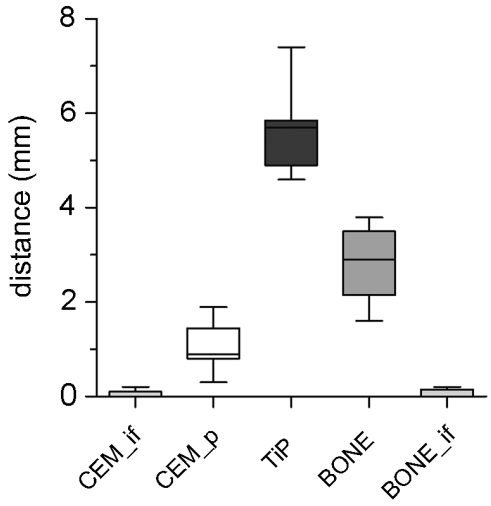


Figure 5 *Histomorphometric quantification (distance in millimeters) of the acetabular reconstruction: **cem_if**: fibrous tissue layer between the bone cement and TiP; **cem_p**: penetration depth of cement into the TiP layer; **TiP**: TiP graft layer thickness; **bone**: bone ingrowth depth in the TiP layer; **bone_if**: thickness of the fibrous tissue layer between TiP and the surrounding bone.*

Wear and systemic titanium concentrations

Before implantation, the articular surface of the cups showed machinery marks, however, no signs of scratching or damage were observed. 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 cups of non-dislocated hips showed diminishing of machinery marks especially in the loaded area, with areas of scratching in most cups. Non-dislocated femoral heads were shiny as preoperatively with some isolated, small and superficial scratches (Figure 6). The two dislocated hip joints showed some wear of the cup, comparable to the other cups. However, the femoral heads looked tarnished at the contact area with the periphery of the reconstruction. Higher magnification views revealed extensive deep scratching from friction with the rim of the metal mesh. Despite the wear of the dislocated femoral heads, no metallosis was observed and cobalt levels were comparable to the other goats. In general, mean whole blood cobalt concentrations were not time dependent, measuring 1.40 (SD 0.37) ppb before operation and 1.46 (SD 0.46) ppb fifteen weeks after operation, p=0.80. 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).

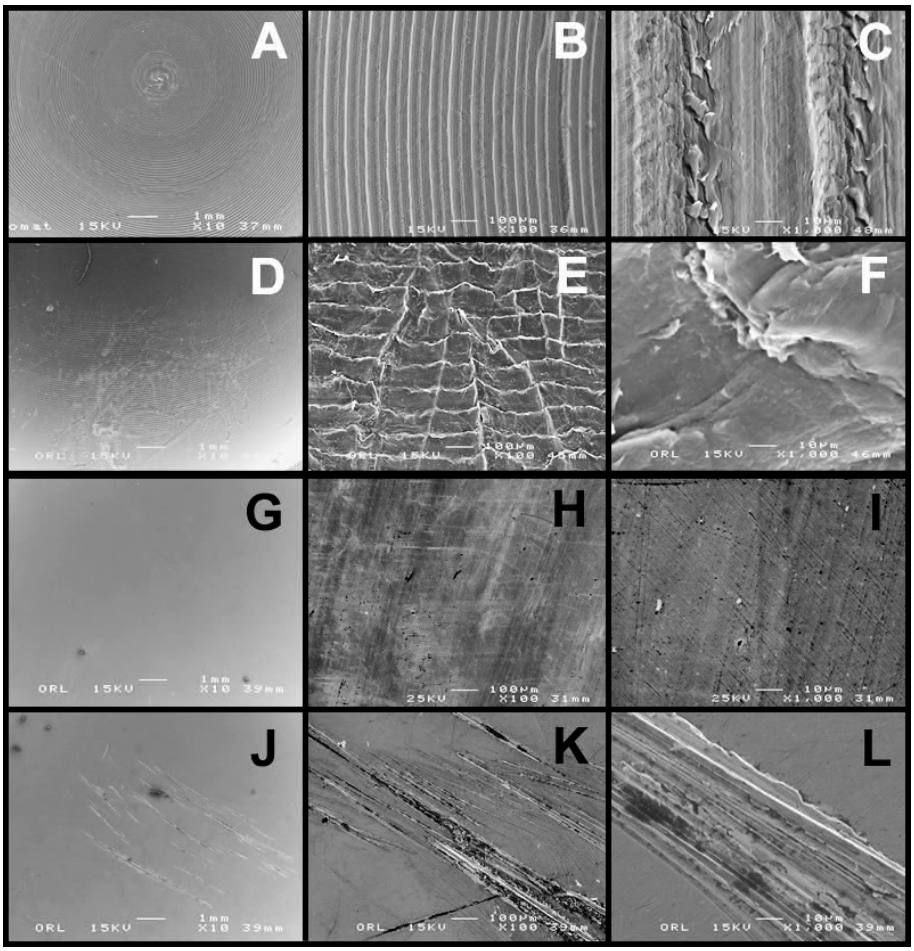


Figure 6 *A-L: Representative views of the cup before (A-C) and after (D-F) implantation and of the femoral head before (G-I) and after (J-L) implantation (scanning electron microscopy, magnification at 10x, 100x and 1000x).*

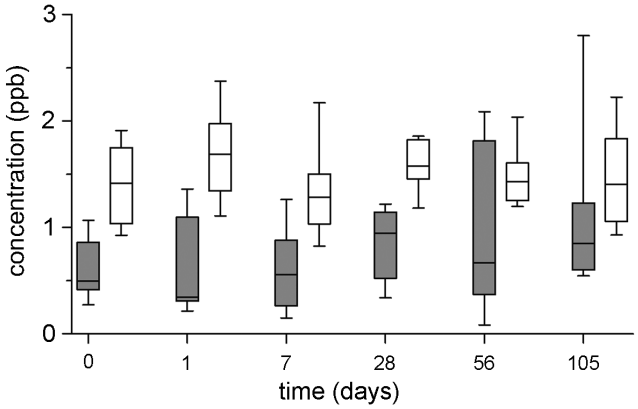


Figure 7 *Time dependence of whole blood concentration (parts per billion) of Cobalt (Co) and Titanium (Ti).*

DISCUSSION

This study focuses on the applicability of TiP for the reconstruction of large acetabular bone defects with impaction grafting and a cemented cup. Calcium phosphate coated TiP showed surgical handling properties comparable to cancellous bone particles. After implantation, all impacted graft layers showed bone armouring in a realistic animal model [31]. The used animal method is quite critical due to several factors. Firstly, full weight bearing was allowed direct postoperatively. Secondly, the goats were relatively old (retired milk goats) which decreases the biological back-up [32]. Additionally, the femoral head was relatively large compared to the cup diameter which causes increased shear stresses within the reconstruction [33]. Despite these challenging conditions, the TiP graft layer itself remained mechanically stable and after fifteen weeks the porous titanium graft layer was osseointegrated with the surrounding bone. Further, a fibrous tissue interface was nearly absent. From a mechanical point of view, the direct interdigitation of bone cement with the non resorbable and osseointegrated graft layer matrix means a rigid fixation of the cement layer to the surrounding host bone within fifteen weeks postoperatively. In contrast, in bone impaction grafting, initially the graft is probably in close contact with the cement but after ingrowth of new tissue and remodeling the incorporated bone graft layer is usually separated from the bone cement by a fibrous tissue interface. This

is probably the end stage of the natural incorporation process of bone grafts were the revascularization and re-ossification process is preceded by a fibrous transition zone, leaving a fibrous tissue interface between the bone grafts and the opposing cement layer [34]. After incorporation of the graft layer, long-term stability is dependent on the fibrous tissue interface between cement and graft layer. It allows micro motion of the cup relative to the surrounding graft layer, leading to osteolysis of the surface of the adjacent bone graft layer and replacement of the lost bone stock by an additional layer of fibrous tissue. This cascade could be responsible for initiation of the loosening process and is in agreement with previous observations after acetabular impaction grafting in the same goat model [31, 35].

Some differences were found compared to previous studies: Firstly, cement penetration was limited in this study compared to an in vitro study in synthetic acetabula with similar TiP. The primary surgeon (BWS) was involved in both studies and timing of cementation was comparable. The main cause responsible for the difference in cement penetration depth seems to be the inferior in vivo pressurizing of the bone cement due to a bad fit of the pressurizer with the reconstructed goat acetabulum compared to the perfect fit with a synthetic and round acetabular sawbone model. The effect of inferior pressurization was detrimental in one goat where the cup failed at the cement-TiP interface. The cement penetration in this reconstruction was negligible (0.3 mm) with no direct contact between the bone cement and the underlying TiP layer over at the majority of the interface. Although inferior pressurizing might be the explanation for failure in this reconstruction, it should also be taken into account that the TiP themselves might be more susceptible to the condition of inferior pressurizing as cement will need higher pressures to penetrate into the micro-porous surface of TiP compared to penetration into the more macro porous surface of bone particles. Secondly, standardization of the amount of impaction is a difficult issue in in vivo impaction grafting. Although the primary surgeon (BWS) was involved in both in-vivo and in-vitro impaction and the impactors were designed to result in a standardized degree of impaction, some variation was unavoidable. The size of the acetabular defect greatly depended on the size of the acetabulum which is highly variable in goats. However, the range of bone ingrowth distances in the different goats suggests that the variation in graft layer porosity was acceptable. Finally, no titanium micro particles were observed in high magnification microscopic views as opposed to previous studies

were ex-vivo impaction was used [27, 36]. The lack of observed particles could be the result of a lower amount of generated particles due to lower in-vivo impaction forces and the lack of friction between TiP and a surrounding metal chamber. In addition, generated micro-particles could have been partly removed during the surgical procedure by fluid currents caused by the impaction procedure itself as well as by subsequent rinsing (pulse lavage).

Despite favorable initial histological characteristics as substantial bony armouring and a minimal fibrous tissue interface, there is still some concern about the long-term viability of the TiP graft layer. In this study, calcium phosphate coated TiP were used and all reconstructions showed profound bone ingrowth. However, from a non loaded in vivo study it was concluded that non coated TiP show only superficial bone ingrowth [29]. The same observation was made in a small loaded pilot study with three goats and non coated TiP in exactly the same goat model as used in this stud y (non published data). Therefore it seems essential to use calcium phosphate coated TiP instead of non coated TiP in order to stimulate bone ingrowth and by that minimize micro motion and subsequent fretting corrosion and fatigue of the TiP graft layer on the longer term. Secondly, it cannot be excluded that fretting wear occurs after prolonged loading by micro motion between individual TiP within the graft layer. It could lead to osteolysis metal and / or polyethylene particle disease [37-39]. During the time scale of this study we did not observe any of these signs. Despite mixed wear patterns with abrasive marks in the surface of the cup, the wear of the cups was insufficient to clear all machinery marks after fifteen weeks. Cups retrieved from a goat study with biphasic calcium phosphate particles as a bone substitute showed a comparable pattern and quantity of wear with also some machinery marks left.[26] It could be hypothesized that the (large quantity of) calcium phosphate debris would cause third body wear of the relatively soft surface of the polyethylene cup as bone debris is capable of causing third body wear of a much harder CoCr surface [38]. However, there is no evidence so far from clinically studies that this third body wear occurs or is clinically relevant [40]. Bearing this in mind the observed wear within fifteen weeks of implantation is not considered to be clinically relevant. In addition, the absolute levels of the titanium blood concentrations at the end of the study (approx. 1 ppb) were as low as the reference values in humans without an implant (approx. 1-2 ppb) and considerably lower than in patients with for instance a loose titanium hip prosthesis (approx. 8 ppb)

[41, 42]. An increase in metal blood concentrations is observed with failing implants and results from metallosis and the subsequent local exposure to a large total surface of metal, increasing local metal ion concentrations. However, the increase in titanium blood concentration is also a known phenomenon in well-functioning titanium hip implants. In this study in goats, the titanium blood concentration rose on a log-linear scale. This means that the concentration is expected to stabilize, probably within concentrations of about 1-2 ppb and might be explained by passive dissolution at the increasing contact area between the implant surface and invading host tissue [42].
Besides the above mentioned issues, other uncertainties remain like the revisability of a TiP graft layer compared to a bone particle graft layer and the long term fate of a reconstructive TiP layer that does not show bony armoring.

Conclusions

TiP with a thin calcium phosphate coating as a full bone substitute material showed good surgical handling in acetabular bone impaction grafting. The impacted TiP layer was osteoconductive under fully loaded conditions. One reconstruction showed minimal cement penetration and failed at the cement-TiP interface. There were no signs of particle generation or accumulation and titanium blood concentrations remained low. From this study it is concluded that calcium phosphate coated TiP seem a promising full bone substitute material for acetabular impaction grafting. TiP should be used with an adequate cementing technique, including pressurizing of the cement. A long term clinical pilot study should be conducted with reconstruction of smaller defects to evaluate the safety and revisability of a TiP reconstruction.

Acknowledgements

Data were collected by Leon Driessen (histomorphometric support, Department of Orthopaedics, Orthopaedic Research Lab, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands) and Marc Uytterhoeven (analysis of metal concentrations, Algemeen Medisch Laboratorium (General Medical Laboratory), Antwerpen, Belgium).

Contributions and disclosure

The study was designed by all authors. TiP (non coated) were provided by Fondel Medical B.V., Rotterdam, The Netherlands. TiP were coated and prepared for implantation by LHBW. Operations were done by BWS, LHBW and RA. Analysis and interpretation of data was done by LHBW and PB. The manuscript was written by LHBW. This study was financed by Fondel Medical B.V., Rotterdam, The Netherlands. The sponsor had no involvement in: the design of the study, collection, analysis and interpretation of data, writing of the manuscript or the decision to submit this manuscript for publication.

REFERENCES

1. Grotle M, Hagen KB, Natvig B, Dahl FA, Kvien TK. Prevalence and burden of osteoarthritis: results from a population survey in Norway. J Rheumatol. 2008;35(4):677-84.
2. Roux CH, Saraux A, Mazieres B, Pouchot J, Morvan J, Fautrel B, et al. Screening for hip and knee osteoarthritis in the general population: predictive value of a questionnaire and prevalence estimates. Ann Rheum Dis. 2008;67(10):1406-11.
3. http://www.datamonitor.com/Products/Free/Report/DMHC2264/010DMHC2264.pdf
4. Baumann C, Rat AC, Osnowycz G, Mainard D, Cuny C, Guillemin F. Satisfaction with care after total hip or knee replacement predicts self-perceived health status after surgery. BMC Musculoskelet Disord. 2009;10:150.
5. Jones CA, Voaklander DC, Johnston DW, Suarez-Almazor ME. Health related quality of life outcomes after total hip and knee arthroplasties in a community based population. J Rheumatol. 2000;27(7):1745-52.
6. Mariconda M, Galasso O, Costa GG, Recano P, Cerbasi S. Quality of life and functionality after total hip arthroplasty: a long-term follow-up study. BMC Musculoskelet Disord. 2011;12:222.
7. Falbrede I, Widmer M, Kurtz S, Schneidmuller D, Dudda M, Roder C. [Utilization rates of lower extremity prostheses in Germany and Switzerland: A comparison of the years 2005-2008]. Orthopäde. 2011;40(9):793-801.
8. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am. 2007;89(4):780-5.
9. Otten R, van Roermund PM, Picavet HS. [Trends in the number of knee and hip arthroplasties: considerably more knee and hip prostheses due to osteoarthritis in 2030]. Ned Tijdschr Geneeskd. 2010;154:A1534.
10. de Kam DC, Gardeniers JW, Hendriks JC, Veth RP, Schreurs BW. Cemented polyethylene cups in patients younger than 40 years. Clin Orthop Rel Res. 2009;467(7):1753-64.
11. Garellick G, Kärrholm J, Rogmark C, Herberts P. Swedish Hip Arthroplasty Register. Annual Report 2008. https://www.jru.orthop.gu.se/ October 2009.
12. Bauer TW. Particles and periimplant bone resorption. Clin Orthop Rel Res. 2002(405):138-43.
13. Wainwright C, Theis JC, Garneti N, Melloh M. Age at hip or knee joint replacement surgery predicts likelihood of revision surgery. J Bone Joint Surg Br. 2011;93(10):1411-5.
14. 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.
15. Flecher X, Parratte S, Brassart N, Aubaniac JM, Argenson JN. Evaluation of the hip center in total hip arthroplasty for old developmental dysplasia. J Arthroplasty. 2008;23(8):1189-96.
16. Gie GA, Linder L, Ling RS, Simon JP, Slooff TJ, Timperley AJ. Impacted cancellous allografts and cement for revision total hip arthroplasty. J Bone Joint Surg Br. 1993;75(1):14-21.
17. Paprosky WG, O'Rourke M, Sporer SM. The treatment of acetabular bone defects with an associated pelvic discontinuity. Clin Orthop Rel Res. 2005;441:216-20.
18. Garcia-Cimbrelo E, Marti-Gonzalez JC. Circular external fixation in tibial nonunions. Clin Orthop Rel Res. 2004(419):65-70.

19. Lee JM, Nam HT. 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.

20. van Haaren EH, Heyligers IC, Alexander FG, Wuisman PI. High rate of failure of impaction grafting in large acetabular defects. J Bone Joint Surg Br. 2007;89(3):296-300.

21. 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.

22. 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.

23. Arts JJ, Schreurs BW, 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 Mech Eng [H]. 2005;219(4):257-63.

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

25. Bolder SB, Verdonschot N, Schreurs BW, 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.

26. Arts JJ, Gardeniers JW, Welten ML, Verdonschot N, Schreurs BW, Buma P. No negative effects of bone impaction grafting with bone and ceramic mixtures. Clin Orthop Rel Res. 2005;438:239-47.

27. Walschot LH, Schreurs BW, Buma P, Verdonschot N. Impactability and time-dependent mechanical properties of porous titanium particles for application in impaction grafting. J Biomed Mat Res Part B Appl Biomater. 2010;95(1):131-40.

28. Walschot LH, Aquarius R, Schreurs BW, 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 Mat Res Part B Appl Biomater. 2013;101(7):1243-50.

29. Walschot LH, Aquarius R, Schreurs BW, 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 Mat Res Part B Appl Biomater. 2012;100(6):1483-9.

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

31. Schimmel JW, Buma P, Versleyen D, Huiskes R, Slooff TJ. 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.

32. 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.

33. Charnley J. Total hip replacement by low-friction arthroplasty. Clin Orthop Rel Res. 1970;72:7-21.

34. 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.

35. De Man FH, Tigchelaar W, Marti RK, Van Noorden CJ, Van der Vis HM. 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.

36. Walschot LH, Schreurs BW, 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.

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

38. Davidson JA, Poggie RA, Mishra AK. 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.

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

40. Blom AW, Wylde V, Livesey C, Whitehouse MR, Eastaugh-Waring S, Bannister GC, et al. Impaction bone grafting of the acetabulum at hip revision using a mix of bone chips and a biphasic porous ceramic bone graft substitute. Acta Orthop. 2009;80(2):150-4.

41. Jacobs JJ, Skipor AK, Black J, Urban R, Galante JO. 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.

42. Jacobs JJ, Skipor AK, Patterson LM, Hallab NJ, Paprosky WG, 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.

CHAPTER EIGHT

SUMMARY AND GENERAL DISCUSSION

The **general introduction (chapter one)** describes subsequently: background issues concerning arthritis of the hip (degradation of the hip joint surface), replacement of the hip joint by an artificial implant (THA: total hip arthroplasty), techniques to deal with associated bone defects, and the idea of assessing the applicability of porous titanium particles (TiP) as a full bone substitute material for the impaction grafting technique to reconstruct bone defects during hip joint replacement surgery (subject of this thesis). The results of the biomechanical in vitro studies and the biological in vivo studies, designed to answer the stated research questions at the end of the general introduction, can be summarized as follows:

1. *Are the handling properties of porous titanium particles sufficient to reconstruct a bone defect in hip joint replacement surgery?*

YES

Chapter two describes the basal biomechanical properties of TiP including impactability, resistance to compressive forces during physiological loading after impaction and elasticity during loading after impaction. TiP were more deformable than bone particles and created a heavily entangled, macro porous graft matrix. After impaction, TiP were very resistant to compressive forces and stiffer than bone particles but more elastic than calcium phosphate particles.

2. *Does impaction or loading of porous titanium particles result in micro particle release?*

YES

Chapter two describes also the evaluation of titanium micro particle generation during impaction and compares the amount and size of the impaction debris particles of TiP, bone and calcium phosphate particles. TiP generated the lowest volume of micro particles which had a bimodal size distribution. 99.8% of the particle volume was too large to be phagocytized (Ø >10 µm). In addition, **chapter three** describes a comparable and low volume of titanium micro particles isolated from graft layers of femoral reconstructions after impaction. The titanium micro particles had a three modal size distribution and 99.5% of the particle volume was too large to be phagocytized (Ø >10 µm). There was

no detectable particle release during subsequent prolonged loading of the femoral reconstructions.

3. *Does a reconstructive layer of porous titanium particles provide adequate primary stability to allow full weight bearing without compromising bone ingrowth?*

YES

The study described in **chapter three** shows substantial initial subsidence of the femoral stem during the first loading cycle, as is often seen in taper slip design stems. After this initial migration, a low and reproducible time-dependent stem subsidence was observed during prolonged loading. **Chapter four** describes the primary stability of realistic acetabular reconstructions. Titanium reconstructions showed four times less residual displacement compared to pure bone particle reconstructions during a short term loading regime. In addition, titanium reconstructions were three to four times more resistant to shear stress.

4. *Is a reconstructive layer of impacted TiP penetrated by bone cement during cementation of a total hip prosthesis?*

YES

In the study described in **chapter three,** cement penetrated 0.49 (SD 0.11) mm in the TiP graft layer of femur reconstructions. The cement penetration in the TiP layer of the acetabulum reconstructions described in **chapter four** was less deep and amounted to 3.8 (SD 0.5) mm**.** Correspondingly the density of TiP layers impacted in the acetabulum was somewhat lower (0.9 g / ml) than TiP layers impacted in the femur (1.1 g / ml). Despite shallower cement penetration in acetabulum TiP reconstructions, compared to acetabulum bone particle reconstructions, TiP reconstructions demonstrated a considerably better primary stability than bone particle reconstructions. The femur TiP reconstructions also showed excellent primary stability despite less deep cement penetration than the acetabulum TiP reconstructions.

5. Are porous titanium particles osteoconductive like allograft bone particles?

YES

Chapter five describes a study to evaluate the osteoconductivity of small TiP (Ø 1.0 – 1.4 mm). Bone growth was observed in the pores between and within TiP, inserted in a titanium bone conduction chamber. However, insertion of TiP as well as insertion of impacted bone particles in the titanium bone conduction chamber decreased bone ingrowth distance in the bone chamber, compared to the bone ingrowth distance in an empty bone chamber. Moreover, smaller bone ingrowth distances were observed in the titanium bone chambers filled with the non resorbable TiP compared to the titanium bone chambers filled with resorbable bone particles. Therefore, it was concluded that the osteoconductivity of TiP might be adversely affected by impaction.

6. Does impaction alter the osteoconductivity of porous titanium particles?

PROBABLY

The non loaded titanium bone conduction chamber study described in **chapter five** showed a trend towards a decrease in bone ingrowth distance if the inserted small TiP (Ø 1.0 – 1.4 mm) were impacted compared to non impacted TiP. However, the effect of impaction was not statistically significant.

7. Does a surface treatment alter the osteoconductivity of porous titanium particles?

YES

Chapter five showed a trend that impacted TiP with a coating of silicated calcium phosphate showed further ingrowth of bone than impacted TiP without a coating. **Chapter six** describes a non loaded in vivo study in goats with four different groups of impacted TiP. Bone ingrowth occurred within four weeks of implantation in all TiP groups. In this study, the non coated TiP showed significantly less bone ingrowth than TiP coated with silicated calcium phosphate or carbonated apatite. Bone ingrowth distance in TiP coated with silicated calcium phosphate or carbonated apatite was comparable to bone ingrowth distance in bone particles and calcium phosphate particles.

8. Do impacted porous titanium particles result in an osseo-integrated implant?

YES

Chapter seven describes a realistic in vivo study in goats to mimic revision arthroplasty of the cup with restoration of a large bone defect. The reconstructive layer of impacted calcium phosphate coated TiP proved to be osteoconductive under loaded conditions. Impacted TiP indeed resulted in an osseo-integrated implant: direct interlocking of the TiP layer was observed both with the bone cement at the side of the implant as well as with the surrounding bone on the other side of the TiP layer. Only a very limited part of the contact surfaces showed a fibrous tissue interface. However, one reconstruction failed at the cement-TiP interface. Retrospective analysis of this reconstruction revealed minimal cement penetration. This observation raises the suspicion that the mechanically weakest link of a cemented TiP impaction grafting reconstruction might be the cement-TiP interface. Early application of bone cement (low viscosity) with adequate pressurizing should optimize cement penetration and thereby prevent debonding at the cement-TiP interface.

9. Does the implantation of porous titanium particles result in third body wear of the artificial articular surface?

QUESTIONABLE

Tribological analysis described in **chapter seven** did not suggest clinically relevant third body wear of the cup within fifteen weeks of implantation. From the available data in **chapter two**, the absolute volume of titanium micro particles seems rather minimal compared to the amount of micro particle release of bone particles and especially compared to calcium phosphate particles. However, 90% of the micro particle volume generated during impaction of TiP consisted of micro particles with a diameter > 100 µm which are expected to cause more tribologic damage than the usual micron sized particles that are generated by fretting corrosion in titanium implants. Long-term evaluation is indicated.

10. Do implantation and in vivo loading of porous titanium particles result in systemically elevated Ti concentrations?

QUESTIONABLE

Chapter seven describes a small though significant rise in titanium concentrations in blood throughout the implantation period of fifteen weeks. After fifteen weeks of implantation the titanium concentrations in blood were comparable to titanium concentrations in blood of healthy people without a titanium orthopedic implant. The increase had a log linear character (slowing down with time). Therefore, the titanium concentrations in blood are expected to remain low in the long-term. However, humans are bipedal in contrast to goats who are quadrupedal. Consequently, a relatively larger volume of TiP will be implanted in humans compared to the applied goat model. Accordingly, the absolute titanium concentration in humans after implantation of TiP for hip joint replacement surgery might exceed reference values (2 ppb) in humans without implants.

GENERAL DISCUSSION

After answering the main questions posed at the end of the general introduction, some topics remain to be discussed with respect to the clinical applicability of TiP in impaction grafting for hip arthroplasty. **Chapter seven** showed that impacted TiP provided sufficient primary stability under loaded conditions in the challenging environment of a large acetabular reconstruction. Further, profound bone ingrowth was observed after fifteen weeks of implantation. Despite these promising results on the acetabular side, no realistic animal model was conducted to evaluate the applicability on the femoral side. Although **chapter three** describes low subsidence rates after 300,000 cycles of in vitro loading, femoral application poses surgical, biomechanical and biological challenges different to acetabular application. Firstly, the entrance to the femoral intra-medullary canal of the goat is relatively narrow. Secondly, despite uneventful in vitro application in the femur and uneventful in vitro as well as in vivo application in the acetabulum, TiP might turn out to be too hard for femoral application: the radially directed forces during impaction with a tapered impactor could result in a fissure or fracture of the usually osteopenic or osteoporotic femur. Further, the often sclerotic inner surface of the femoral shaft is biomechanically disadvantageous as it provides less interlocking with the TiP compared to the more cancellous structure of acetabular bone. Finally, there might be a biological challenge on the femoral side as bone graft incorporation is generally less than on the acetabular side. On the other side, these femoral conditions are also the same in the patients who have undergone femoral impaction grafting with bone grafts. Despite the less attractive circumstances, very satisfying long-term outcome has been reported after femoral bone impaction grafting.

A very basic question concerns the standardization of the geometrical and mechanical properties of the implanted TiP. TiP are made from a bulk material with a wide range in size, porosity, pore sizes and micro stucture. From this bulk material, the TiP as applied in the studies described in this thesis were selected based on size, specific weight (porosity volume percentage) and macroscopic appearance. The production and selection method needs to be improved with respect to standardization of the presented fully synthetic potential grafting material of TiP. Mechanical properties (impactability and elasticity)

and biological properties (osteoconductivity) of TiP will depend on the porosity, pore size distribution, pore fenestration size and surface characteristics [1-6]. Therefore, it is strongly advisable to standardize these parameters within certain limits before introduction of the clinical application of TiP in impaction grafting in order to guarantee a standardized product with predictable biomechanical and osteoconductive properties [2-6]. With respect to the osteconductivity, especially the pore size distribution of a matrix of TiP is of interest as concluded in **chapters five and six** were different particle sizes (1.0 – 1.4 mm and 2.8 – 4.0 mm) were implanted. Histologic observations showed smaller pore diameters in the smaller particles (1.0 – 1.4 mm) compared to the larger particles (2.8 – 4.0). Bone ingrowth depended on the presence of the larger inter-particle pores (measuring approximately 50 to 200 µm). As a result, the larger particles performed considerably better than the smaller particles with respect to osteoconductivity. These observations should be taken into consideration together with the recommendations in the literature that optimal pore sizes for osteoconduction range between 100 to 400 µm. Therefore, ''pore patency'' (pore fenestration size) should be at least 100 µm. In this respect it should be considered that impaction of TiP will have an effect on both the pore size distribuation and pore fenestration size within individual TiP particles as well as between different TiP particles [2-6]. Alternative techniques to standardize the above mentioned geometric and mechanical parameters of a porous titanium matrix might be spraying, printing or a polymeric sponge replication process [1,7,8].

The advise to standardize the above mentioned parameters of porous titanium in order to optimize osteoconductivity raises the question if bony armouring is necessary at all, a question that can not readily be answered with the available literature and data presented in this thesis. The assumption that bony armouring would be necessary, is supported by the hypothesis that the cascade of loosening is initiated by a (thin) soft tissue interface between the implanted graft and the bone cement or the surrounding bone. This soft tissue interface will not be present with an osseo-integrated implant [9]. Further, bony armouring is expected to result in a stiffer reconstructive layer than fibrous armouring, thereby reducing the potential of by loading induced micro motions with subsequent fretting wear at the surface of interdigitating protruding parts of individual TiP particles. For these reasons, it seems preferable to aim for optimal osteoconductivity by a surface treatment to overcome the limited osteoconductive properties of non

coated TiP. From this view point an interesting technique should be mentioned which is completely different from application of a calcium phosphate coating: Takemoto et al described that chemically roughening of porous commercially pure titanium implants can create an osteoinductive implant. Porous titanium cylinders treated with an alkaline solution, dilute HCl, hot water and heat resulted in a combined micro and macro porosity and osteoinduction in all implants [8,10]. The advantage of applying such a technique is that the surface treatment effect is durable in time as opposed to application of a bioresorbable coating like carbonated apatite or hydroxyapatite.

Besides the above mentioned issues, some other aspects might be relevant. Firstly, as opposed to conventional implanted materials like bone particles and calcium phosphate particles, TiP are non resorbable. Consequently, the reconstructive layer will not be mechanically jeopardized by resorption. On the other hand a non resorbable material might be subject to fatigue. However, titanium is known for it's high fatigue resistance. Trabecular Metal® has been used for manufacturing of pre-shaped augments and is also made of a highly porous biocompatible metal (tantalum). These augments have been successfully used as weight bearing gap fillers in both primary as well as revision arthroplasty. So far, no fatigue failure has been observed after prolonged in vivo loading [11].

Secondly, infection of a non resorbable material like TiP might create the need for additional surgical procedures like a two stage revision. However, with respect to this, the application of allograft bone particles should not necessarily be considered to be advantageous compared to the application of porous metal particles. Like metal, allografts act like a devascularized foreign body and are also quickly covered by a biofilm in case of a deep infection [12]. Impregnation of calcium phosphate coated TiP with a potent inhibitor (vancomycine, silver) of colonization by pathogens probably make TiP even less susceptible to infection than non impregnated allograft bone particles [12-14].

Thirdly, the phenomenon of galvanic corrosion might occur with implantation of a metal implant. Although this issue should be briefly mentioned, the concern about galvanic corrosion does not seem to be well founded. Orthopedic implants constituted of different metals and in direct or indirect contact with each other have been implanted for many years. So far, galvanic corrosion has not been a clinically relevant item even not with

the implantation of the highly porous Trabecular Metal® augments, which have a large specific area comparable to TiP. Further, commercially pure titanium is a chemically inert material due to rapid passive oxidation. For these reasons, implantation of TiP in direct contact with, or in the vicinity of another metal (for instance a CoCr metal mesh or a CoCr hip prosthesis) is not expected to be associated with galvanic reactions of any significance [15,16].

Another question refers to one of the primary goals of defect reconstruction by impaction grafting and concerns the future revisability of a reconstructive layer of impacted TiP. A graft layer of impacted autograft or allograft bone particles will be incorporated and converted into new host bone within one or two years postoperatively. It is questionable if a reconstructive layer of TiP, armoured with fibrous tissue and / or host bone is machinable with conventional surgical tools like a reamer and a broach. From industrial applications like aircraft industry it is known that cp titanium (tensile strength 241 to 550 MPa) machines much easier than titanium alloys (tensile strength up to 1380 MPa). Additionally to low speed reaming, which is usual in the operating theatre, conditions like sharp instruments and excessive cooling with water seem prerequisites to prevent smearing and galling of the cp titanium.

Finally, this thesis describes the application of TiP with a *cemented* technique. Although good clinical results can be found in the literature for acetabular bone impaction grafting with both a cemented as well as an uncemented technique, we do not advise to combine impaction grafting with porous titanium and an uncemented cup [17,18]. During in vitro loading of a TiP impaction grafting reconstruction (combined segmental and cavitary defect) with an uncemented metal backed cup, the cup showed progressive migration. This resulted in early failure of the initially press fit fixated cup. The associated plastic deformation of the TiP layer is probably due to high local stressess in the surface of the TiP layer where it comes into contact with the relatively rigid metal backed cup. Mechanically, this is a totally different situation from the gradual decrease in stiffness with a cemented cup where bone cement penetrates into the surface of the TiP layer. The progressive plastic deformation of the TiP layer as a result of loading of the metal backed cup resulted in early failure of the initially press fit fixed cup (unpublished data).

In accordance with the advice not to apply TiP as a weight bearing graft layer in an uncemented arthroplasty, it might be logical to apply a similar strategy with the

mechanically even weaker and more deformable bone particles. The insertion of a cement layer between the hard metal cup and the relatively soft graft layer of impacted TiP would distribute the compressive forces over a much larger graft layer area, leading to stress levels below the failure level of the titanium graft layer. The good clinical results of uncemented acetabular bone impaction grafting were observed mainly in cavitary defects [18]. In the absence of a substantial segmental defect, a well fixated press fit cup will decrease the loading of the underlying bone particle graft layer compared to the situation of a combined segmental and cavitary defect. Consequently, application of TiP or bone particles as a gap filler might be defendable in uncemented reconstruction of a smaller cavitary defect.

In conclusion, in the conducted studies, calcium phosphate coated TiP meet the primary demands of a full bone substitute material for cemented acetabular impaction grafting. TiP are deformable like bone particles and therefore possess favorable handling characteristics. During the operation, TiP can be impacted and shaped by the surgeon according to the encountered (highly variable) osseous defect dimensions and the dimensions of the implanted prosthesis. This is a considerable advantage compared with other porous metal gap fillers (mainly Trabecular Metal®) currently available on the market: these augments are pre-shaped, do not adept to the defect geometry and are not available as deformable particles [11]. After impaction, TiP create a macro porous graft layer able to withstand direct postoperative full load bearing.

A graft layer of impacted TiP is armoured by ingrowth of bone within fifteen weeks during direct postoperative full load bearing. A very small amount of titanium micro particles is released during impaction of the TiP. However, after impaction, there were no signs of titanium micro particle release during prolonged loading. There were no signs of accelerated wear of the polyethylene cup within 15 weeks of loading. Although titanium blood concentrations increased significantly, absolute values remained very low.

At the end of this thesis, suggestions for further pre-clinical research as well as the settings of a human pilot study are formulated:

In order to optimize osteoconductivity, the diameter of the TiP should measure at least 2.8 – 4.0 mm and TiP should be calcium phosphate coated. In order to maintain osteoconductivity after (firm) impaction, pores between impacted particles (inter-

particle pores), and preferentially also within impacted particles (intra-particle pores) should measure at least 100 µm. Further pre-clinical evaluation of application of TiP as a full bone substitute material for cemented *femoral* impaction grafting should consist of a realistic loaded in vivo study with reconstruction of a large femoral defect in an adequate animal model (goat or sheep), similar to the acetabular reconstruction. Attention should be paid to apply a proper cementation technique with early introduction of the cement and adequate pressurizing to guarantee sufficient interdigitation of the bone cement and the surface of the impacted titanium graft layer.

In order to progress to utilization of these titanium particles on a larger scale, we recommend to conduct a prospective human pilot study prior to this. Within this human pilot study smaller acetabular (AAOS type I and II) and femoral ((Valle and Paprosky type I and II) defects should be reconstructed by surgeons experienced in the impaction grafting technique [19,20]. This pilot study should show whether the application of TiP is safe in humans in the long-term with respect to the fracture risk in osteopenic and osteoporotic bone, mechanical reconstruction stability, third body wear, fretting corrosion and fatigue, absence of adverse tissue reactions, stabilization of titanium blood concentrations and revisability. After successful introduction of TiP in cemented impaction grafting of smaller defects, the application can be extended to a pilot study with reconstruction of larger defects (AAOS type III and IV, Valle and Paprosky type III).

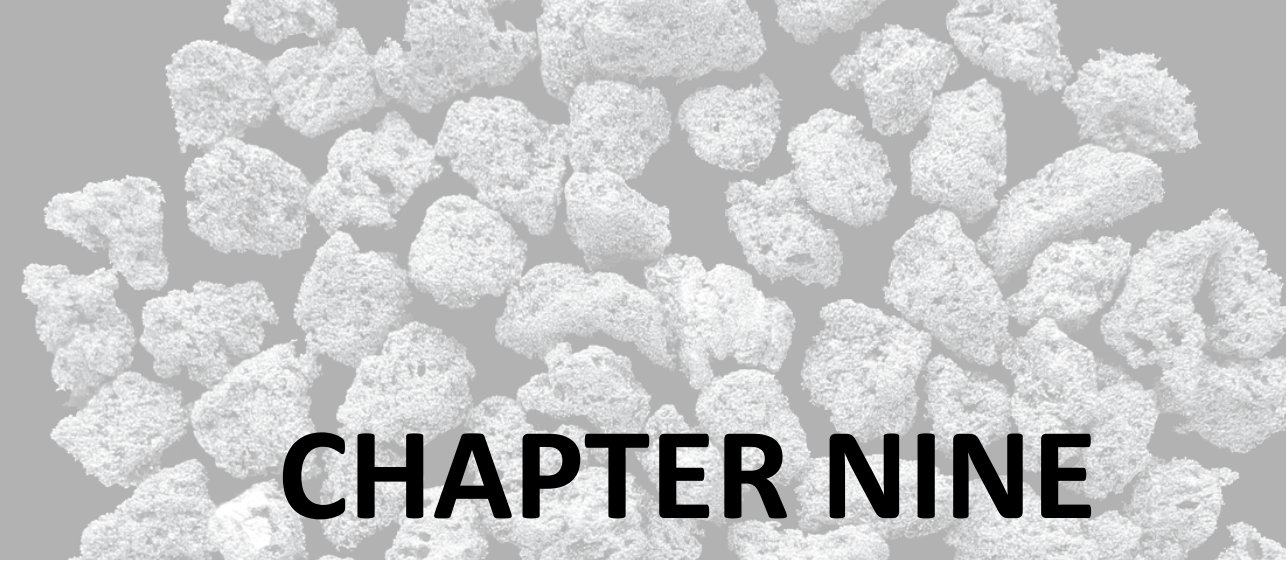
REFERENCES

1. Li JP, Li SH, Van Blitterswijk CA, de Groot K. Cancellous bone from porous Ti6Al4V by multiple coating technique. J Mater Sci Mater Med 2006;17(2):179-85.
2. Bobyn JD, Pilliar RM, Cameron HU, Weatherly GC. The optimum pore size for the fixation of porous-surfaced metal implants by the ingrowth of bone. Clin Orthop Relat Res 1980(150):263-70.
3. Chang BS, Lee CK, Hong KS, Youn HJ, Ryu HS, Chung SS, Park KW. Osteoconduction at porous hydroxyapatite with various pore configurations. Biomaterials 2000;21(12):1291-8.
4. Itala AI, Ylanen HO, Ekholm C, Karlsson KH, Aro HT. Pore diameter of more than 100 microm is not requisite for bone ingrowth in rabbits. J Biomed Mater Res 2001;58(6):679-83.
5. Jones AC, Arns CH, Hutmacher DW, Milthorpe BK, Sheppard AP, Knackstedt MA. The correlation of pore morphology, interconnectivity and physical properties of 3D ceramic scaffolds with bone ingrowth. Biomaterials 2009;30(7):1440-51.
6. Welsh RP, Pilliar RM, Macnab I. Surgical implants. The role of surface porosity in fixation to bone and acrylic. J Bone Joint Surg Am 1971;53(5):963-77.
7. Basalah A, Shanjani Y, Esmaeili S, Toyserkani E. Characterizations of additive manufactured porous titanium implants. J Biomed Mater Res B Appl Biomater 2012;100B(7):1970-9.
8. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. Biomaterials 2005;26(30):6014-23.
9. De Man FH, Tigchelaar W, Marti RK, Van Noorden CJ, Van der Vis HM. 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.
10. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Matsushita T, Kokubo T, Nakamura T. Osteoinductive porous titanium implants: effect of sodium removal by dilute HCl treatment. Biomaterials 2006;27(13):2682-91.
11. Sporer SM, Della Valle C. Porous metal augments: big hopes for big holes. Orthopedics 2010;33(9):651.
12. Ketonis C, Barr S, Adams CS, Hickok NJ, Parvizi J. Bacterial colonization of bone allografts: establishment and effects of antibiotics. Clin Orthop Relat Res 2010;468(8):2113-21.
13. Radin S, Campbell JT, Ducheyne P, Cuckler JM. Calcium phosphate ceramic coatings as carriers of vancomycin. Biomaterials 1997;18(11):777-82.
14. Noda I, Miyaji F, Ando Y, Miyamoto H, Shimazaki T, Yonekura Y, Miyazaki M, Mawatari M, Hotokebuchi T. Development of novel thermal sprayed antibacterial coating and evaluation of release properties of silver ions. J Biomed Mater Res B Appl Biomater 2009;89(2):456-65.
15. Hol PJ, Molster A, Gjerdet NR. Should the galvanic combination of titanium and stainless steel surgical implants be avoided? Injury 2008;39(2):161-9.
16. Meyer H, Mueller T, Goldau G, Chamaon K, Ruetschi M, Lohmann CH. Corrosion at the cone/taper interface leads to failure of large-diameter metal-on-metal total hip arthroplasties. Clin Orthop Relat Res 2012;470(11):3101-8.
17. Wilson MJ, Whitehouse SL, Howell JR, Hubble MJ, Timperley AJ, Gie GA. The Results of Acetabular Impaction Grafting in 129 Primary Cemented Total Hip Arthroplasties. J Arthroplasty 2013.

18. Lee JM, Nam HT. 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.

19. D'Antonio JA, Capello WN, Borden LS, Bargar WL, Bierbaum BF, Boettcher WG, Steinberg ME, Stulberg SD, Wedge JH. Classification and management of acetabular abnormalities in total hip arthroplasty. Clin Orthop Relat Res 1989(243):126-37.

20. Valle CJ, Paprosky WG. Classification and an algorithmic approach to the reconstruction of femoral deficiency in revision total hip arthroplasty. J Bone Joint Surg Am 2003;85-A Suppl 4:1-6.



CHAPTER NINE

SAMENVATTING EN OVERKOEPELENDE DISCUSSIE

De overkoepelende introductie (hoofdstuk één) beschrijft achtereenvolgens: achtergrond onderwerpen betreffende artritis van de heup (degeneratie van het gewrichtsoppervlak van de heup), vervanging van het heupgewricht door een kunstmatig implantaat (THA: totale heup arthroplastiek), hoe om te gaan met de geassocieerde bot defecten en het idee om poreuze titanium korrels (TiP) toe te passen als een volledig bot vervangend materiaal voor de impaction grafting techniek om bot defecten te reconstrueren tijdens heup gewricht vervangende chirurgie (onderwerp van dit proefschrift). De resultaten van de biomechanische in vitro studies en de biologische in vivo studies, ontworpen om de aan het einde van de overkoepelende introductie gestelde onderzoeksvragen te beantwoorden, kunnen als volgt worden samengevat:

1. *Is de hanteerbaarheid van poreuze titanium korrels dusdanig dat daarmee een bot defect bij heup gewricht vervangende chirurgie gereconstrueerd kan worden?*

JA

Hoofdstuk twee beschrijft de basale biomechanische eigenschappen van TiP waaronder impacteerbaarheid, bestendigheid tegen druk tijdens fysiologische belasting na impactie en de elasticiteit tijdens belasting na impactie. TiP waren meer vervormbaar dan bot korrels en creëerden een macro poreuze matrix van stevig in elkaar verhaakte korrels. Na impactie waren TiP zeer goed bestand tegen druk krachten en stijver dan bot korrels maar elastischer dan korrels van calcium fosfaat.

2. *Resulteert impacteren of belasten van poreuze titanium korrels in het vrijkomen van micro partikels?*

JA

Hoofdstuk twee beschrijft eveneens de evaluatie van de productie van titanium micro partikels tijdens impactie en vergelijkt de hoeveelheid en omvang van het impactie gruis van korrels van poreus titanium, bot en calcium fosfaat. TiP genereerden het laagste volume aan micro partikels welke een bimodale diameter distributie vertoonden. 99,8% van het gruis volume bestond uit deeltjes die te groot zijn voor fagocytose (partikel Ø > 10 µm). Aanvullend hierop beschrijft **hoofdstuk drie** een vergelijkbaar en klein volume

titanium micro partikels, geïsoleerd uit de korrel laag van femur reconstructies direct na impactie. De titanium micro partikels hadden een trimodale diameter distributie en 99,5% van het partikel volume was te groot (partikel Ø >10 µm) om gefagocyteerd te worden. Vervolgens werden de femur reconstructies belast. Tijdens belasten konden geen micro-partikels gedetecteerd worden.

3. *Biedt een reconstructieve laag van poreuze titanium korrels voldoende primaire stabiliteit voor bot ingroei onder volledige belasting?*

JA

Het onderzoek beschreven in **hoofdstuk drie** toont een aanzienlijke initiële zakking van de femur steel tijdens de eerste belasting cyclus, zoals vaak wordt gezien bij het concept van een tapse glijdende steel. Na deze initiële ''zetting'' trad een lage en reproduceerbare, tijdsafhankelijke zakking van de steel op tijdens belasten. **Hoofdstuk vier** beschrijft de primaire stabiliteit van realistische acetabulaire reconstructies. Titanium reconstructies toonden vier maal minder verplaatsing dan reconstructies volledig gemaakt met bot korrels tijdens kortdurende belasting. Verder waren titanium reconstructies drie tot vier maal beter bestand tegen schuif spanning.

4. *Vindt cement penetratie plaats in een laag geïmpacteerde poreuze titanium korrels tijdens cementeren van een totale heup prothese?*

JA

In het onderzoek beschreven in **hoofdstuk drie** penetreerde cement 0.49 (SD 0.11) mm in de TiP laag van femur reconstructies. De cement penetratie in de TiP laag van de acetabulum reconstructies beschreven in **hoofdstuk vier** was minder diep en bedroeg 3.8 (SD 0.5) mm. Hiermee komt overeen dat TiP lagen geïmpacteerd in het acetabulum een lagere dichtheid (0.9 g/ml) hadden dan TiP lagen geïmpacteerd in het femur (1.1 g/ml). Ondanks een minder diepe cement penetratie in acetabulum TiP reconstructies, in vergelijking met acetabulum bot korrel reconstructies, toonden de titanium reconstructies een aanzienlijk betere primaire stabiliteit dan de bot korrel reconstructies. De femur TiP reconstructies toonden eveneens een uitstekende primaire stabiliteit ondanks een minder diepe cement penetratie dan de acetabulum TiP reconstructies.

5. Zijn poreuze titanium korrels osteoconductief zoals allograft bot korrels?

JA

Hoofdstuk vijf beschrijft een onderzoek om de osteoconductiviteit van kleine TiP (Ø 1.0 – 1.4 mm) te evalueren. Na plaatsing in een botgeleidingskamer werd bot ingroei gezien in zowel poriën tussen TiP (*inter*-korrel poriën) als in de poriën binnen in TiP (*intra*-korrel poriën. Echter, zowel het plaatsen van TiP als het plaatsen van geïmpacteerde bot korrels in de titanium botgeleidingskamer verminderden de bot ingroei afstand in de botkamer, vergeleken met de bot ingroei in een lege botkamer. Aanvullend werden kleinere bot ingroei afstanden gezien in botkamers gevuld met de niet oplosbare TiP vergeleken met de bot ingroei afstanden in botkamers gevuld met de oplosbare bot korrels. Derhalve werd geconcludeerd dat impactie een negatief effect zou kunnen hebben op de osteoconductiviteit van TiP.

6. Heeft impactie een effect op de osteoconductiviteit van poreuze titanium korrels?

WAARSCHIJNLIJK

De onbelaste titanium botgeleidingskamer beschreven in hoofdstuk vijf toonde een trend tot verminderde bot ingroei afstand wanneer de erin geplaatste kleine TiP (Ø 1.0 – 1.4 mm) geïmpacteerd werden vergeleken met niet geïmpacteerde TiP. Echter, het effect van impactie was niet statistisch significant.

7. Verandert een oppervlakte behandeling de osteoconductiviteit van poreuze titanium korrels?

JA

Hoofdstuk vijf toont een trend dat geïmpacteerde TiP met een coating van calcium fosfaat en silicaat diepere bot ingroei toonde dan geïmpacteerde TiP zonder coating. **Hoofdstuk zes** beschrijft een onbelaste in vivo studie in geiten met vier verschillende groepen geïmpacteerde TiP. In deze studie toonden de niet gecoate TiP significant minder bot ingroei dan TiP gecoat met calcium fosfaat met silicaat of TiP gecoat met

gecarboneerd apatiet. Bot ingroei afstand in TiP gecoat met calcium fosfaat met silicaat of gecarboneerd apatiet was vergelijkbaar met bot ingroei afstand in allograft bot korrels en calcium fosfaat korrels.

8. Resulteren poreuze titanium korrels in een osseo-geïntegreerd implantaat?

JA

Hoofdstuk zeven beschrijft een realistische in vivo studie in geiten om een cup revisie na te bootsen met gelijktijdige reconstructie van een groot bot defect. De reconstructieve laag van geïmpacteerde calcium fosfaat gecoate TiP bewees osteoconductief te zijn onder volledige belasting. Geïmpacteerde TiP resulteerden inderdaad in een osseo-geïntegreerd implantaat: er bestond zowel een directe vergrendeling van de TiP laag met het bot cement aan de implantaat zijde als een directe vergrendeling met het omgevende bot aan de andere zijde van de TiP laag. Slechts een zeer beperkt deel van het contact oppervlak toonde een dunne bindweefsel laag (interface) tussen de TiP laag en het aangrenzende cement of bot. Echter, één reconstructie faalde op de cement-TiP overgang. Retrospectieve analyse van deze reconstructie toonde minimale cement penetratie in de TiP laag. Deze waarneming doet de verdenking ontstaan dat de mechanisch zwakste schakel van een gecementeerde TiP impaction grafting reconstructie de cement-TiP overgang zou kunnen zijn. Vroeg aanbrengen van bot cement (lage viscositeit) met een adequate druk zou de cement penetratie moeten optimalizeren en daarmee loslating op het niveau van de cement-TiP overgang voorkomen.

9. Resulteert de impactie van poreuze titanium korrels in ''derde lichaam slijtage'' van het scharnierende oppervlak van het kunstgewricht?

TWIJFELACHTIG

Tribologische analyse beschreven in **hoofdstuk zeven** suggereerde geen klinisch relevante ''derde lichaam'' slijtage van de cup binnen vijftien weken na implantatie. Op basis van de beschikbare data in **hoofdstuk twee**, lijkt het absolute volume aan titanium micro-partikels nogal minimaal vergeleken met de mate van micro-partikel vrijgave door bot korrels, met name vergeleken met calcium fosfaat korrels. Echter, 90% van het micro

partikel volume gegenereerd tijdens impactie van TiP bestond uit micro partikels met een diameter > 100 µm welke waarschijnlijk meer tribologische schade veroorzaken dan de gebruikelijke, micron grote partikels welke gegenereerd worden door wrijving bij titanium implantaten. Lange termijn evaluatie is geïndiceerd.

10. Resulteren implantatie en in vivo belasting van poreuze titanium korrels in systemisch verhoogde titanium concentraties?

TWIJFELACHTIG

Hoofdstuk zeven beschrijft een kleine doch significante toename in titanium concentraties in het bloed gedurende de implantatie periode van vijftien weken. Na vijftien weken implantatie waren de titanium concentraties in bloed vergelijkbaar met titanium concentraties in het bloed van gezonde mensen zonder een titanium orthopedisch implantaat. De toename had een log lineair karakter (vermindering in toename snelheid naarmate de tijd vordert). Derhalve valt te verwachten dat de titanium concentraties op lange termijn laag zullen blijven. Echter, mensen zijn tweevoeters in tegenstelling tot geiten. Bijgevolg zal een relatief groter volume TiP worden geïmplanteerd bij mensen vergeleken met het toegepaste geitenmodel. Dienovereenkomstig zou de absolute titanium concentratie bij de mens, na implanteren van TiP bij heupgewricht vervangende operaties, de referentiewaarden bij mensen zonder implantaten (2 ppb) kunnen overschrijden.

OVERKOEPELENDE DISCUSSIE

Na het beantwoorden van de hoofdvragen gesteld aan het einde van de overkoepelende introductie, blijven enkele onderwerpen over om te bediscussiëren met betrekking tot de klinische toepasbaarheid van TiP in impaction grafting bij kunstheupen. **Hoofdstuk zeven** toonde dat geïmpacteerde TiP voldoende primaire stabiliteit boden onder belaste omstandigheden in de uitdagende omgeving van een grote acetabulaire reconstructie. Verder werd diepe bot ingroei gezien na vijftien weken implantatie. Ondanks deze veel belovende resultaten aan de acetabulaire zijde is geen realistisch diermodel gebruikt om de toepasbaarheid aan de femorale zijde te evalueren. Hoewel **hoofdstuk drie** lage inzak waarden liet zien na 300.000 cycli in vitro belasting, vormt de femorale toepassing een andere chirurgische, biomechanische en biologische uitdaging dan toepassing aan de acetabulaire zijde. Op de eerste plaats is de toegang tot het intra-medullaire kanaal van het geiten femur relatief smal. Ten tweede zouden TiP, ondanks ongecompliceerde in vitro toepassing in het femur en ongecompliceerde in vitro en in vivo toepassing in het acetabulum, te hard kunnen blijken te zijn voor femorale toepassing: de radiaal gerichte krachten tijdens impactie met een tapse impactor zouden kunnen resulteren in een fissuur of fractuur van het gebruikelijkerwijze osteopene of osteoporotische femur. Verder is het vaak sclerotische oppervlak van de binnenzijde van het femur biomechanisch nadelig aangezien het minder verankering met de TiP verschaft vergeleken met de meer spongieuze structuur van acetabulair bot. Tot slot zou er zich een biologische uitdaging voor kunnen doen aan de femorale zijde daar bot korrel incorporatie in het femur gewoonlijk minder goed is dan aan de acetabulaire zijde. Aan de andere kant zijn deze femorale condities het zelfde in patiënten die femorale impaction grafting hebben ondergaan met bot korrels. Ondanks de minder aantrekkelijke omstandigheden zijn zeer bevredigende lange termijn uitkomsten gerapporteerd na femorale impaction grafting.

Een zeer basale vraag betreft de standaardisering van de geometrie en mechanische eigenschappen van te implanteren TiP. TiP worden gemaakt van een bulk materiaal met een wijde variatie in grootte, porositeit, porie grootte en micro structuur. Van dit bulk materiaal werden de TiP zoals toegepast in dit proefschrift geselecteerd op basis van afmeting, soortelijk gewicht (volume percentage porositeit) en macroscopisch

aspect. De productie en selectie methoden behoeven verbetering met betrekking tot standaardisatie van het in dit proefschrift gepresenteerde volledig synthetische potentiële transplantaat materiaal. Mechanische eigenschappen (impacteerbaarheid en elasticiteit) en biologische eigenschappen (osteoconductiviteit) van TiP zullen namelijk afhankelijk zijn van porositeit, porie grootte verdeling, gemeenschappelijke porie venster grootte en oppervlakte karakteristieken [1-6]. Het is derhalve sterk aan te raden om deze parameters te standaardiseren binnen bepaalde grenzen voor introductie van de klinische toepassing van TiP in impaction grafting om een gestandaardiseerd product te garanderen met voorspelbare biomechanische en osteoconductieve eigenschappen [2-6]. Met betrekking tot de osteoconductiviteit is met name de porie grootte verdeling van een matrix van TiP van belang zoals geconcludeerd in **hoofdstuk vijf en zes** waar korrels van verschillende grootte (1.0 – 1.4 mm and 2.8 – 4.0 mm) werden geïmplanteerd. Histologische observaties toonden kleinere porie diameters in de kleinere korrels (1.0 – 1.4 mm) vergeleken met de grotere korrels (2.8 – 4.0 mm). Bot ingroei was afhankelijk van de aanwezigheid van de grotere poriën tussen korrels (afmeting ongeveer 50 tot 200 µm). Dien ten gevolge deden de grotere korrels het aanzienlijk beter dan de kleinere korrels wat betreft osteoconductiviteit. Deze waarnemingen zouden in overweging genomen dienen te worden, samen met aanbevelingen uit de literatuur dat de optimale porie afmeting voor osteoconductie tussen de 100 en 400 µm bedraagt. De ''porie doorgankelijkheid'' (gemeenschappelijke porie venster grootte) zou daarmee minimaal 100 µm moeten bedragen. Hierbij dient te worden meegenomen dat impactie van TiP zowel een effect zal hebben op de porie grootte verdeling en porie doorgankelijkheid van poriën binnen in individuele TiP korrels als poriën tussen de verschillende TiP korrels [2-6]. Alternatieve technieken om de hierboven genoemde geometrische en mechanische parameters van een poreuze titanium matrix te standaardiseren zouden sprayen, printen of een polymeer spons replicatie proces kunnen zijn [1,7,8].

Het advies om de hierboven genoemde parameters te standaardiseren voor poreus titanium met het oog op optimaliseren van osteoconductiviteit doet de vraag rijzen of benige wapening überhaupt noodzakelijk is, een vraag die niet zomaar beantwoord kan worden met de beschikbare literatuur en de gegevens gepresenteerd in dit proefschrift. De aanname dat benige wapening noodzakelijk zou zijn, wordt gesteund door de hypothese dat de cascade van loslating wordt geïnitieerd door een (dunne) weke delen interface

tussen het geïmplanteerde transplantaat en de cement laag of het omgevende bot. Deze weke delen interface zal niet aanwezig zijn bij een osseo-geïntegreerd implantaat [9]. Verder kan verwacht worden dat benige wapening resulteert in een stijvere reconstructie dan wapening door bindweefsel, waardoor het risico vermindert op wrijvingsslijtage tijdens door belasting geïnduceerde micro bewegingen van het oppervlak van de in elkaar verstrengelde, uitstekende delen van de individuele korrels. Om deze redenen lijkt het de voorkeur te hebben om optimale osteoconductiviteit na te streven door middel van een oppervlakte behandeling om de beperkte osteoconductieve eigenschappen van niet gecoate TiP te overwinnen. Vanuit dit oogpunt zou de volgende interessante techniek vermeld dienen te worden die geheel verschilt van het aanbrengen van een calcium fosfaat coating: Takemoto et al. beschreven dat het chemisch opruwen van poreuze commercieel pure titanium implantaten een osteoinductief implantaat creëert. Poreuze titanium cylinders behandeld met een alkalische oplossing, verdund HCl, heet water en hitte resulteerden in een gecombineerde macro en micro porositeit en osteoinductie in alle implantaten [8,10]. Het voordeel van het toepassen van een dergelijke techniek is dat het oppervlakte behandelingseffect langdurig is in tegenstelling tot een bioresorbeerbare coating zoals gecarboneerd apatiet of hydroxyapatiet.

Naast de bovengenoemde onderwerpen zouden andere aspecten relevant kunnen zijn. Op de eerste plaats zijn TiP niet oplosbaar, in tegenstelling tot gangbare geïmplanteerde materialen zoals bot korrels en calcium fosfaat korrels. Derhalve zal de reconstructieve laag niet onderhevig zijn aan mechanische verzwakking door resorptie. Aan de andere kant kan een niet resorbeerbaar materiaal onderhevig zijn aan vermoeiing. Titanium is echter bekend om zijn hoge vermoeiingsweerstand. Trabecular Metal® is gebruikt voor het maken van voorgevormde transplantaten en is eveneens vervaardigd uit zeer poreus biocompatibel metaal (tantalum). Deze transplantaten zijn succesvol toegepast als belaste defect vullers bij zowel primaire als revisie kunstgewrichten. Tot dusverre is falen ten gevolge van vermoeiing niet waargenomen na langdurig in vivo belasten [11].
Op de tweede plaats zou infectie van een niet resorbeerbaar materiaal als TiP de behoefte aan additionele chirurgische procedures zoals een two-stage revisie kunnen creëeren. Echter, met het oog hierop zou de toepassing van allograft bot korrels niet noodzakelijkerwijze als voordeling beschouwd dienen te worden vergeleken met

toepassing van poreuze metalen partikels. Net als metaal vormen allografts een niet gevascularizeerd vreemd lichaam en zijn eveneens snel bekleed met een biofilm in het geval van een infectie [12]. Impregnatie van calcium fosfaat gecoate TiP met een krachtige remmer (vancomycine, zilver) van kolonisatie door pathogenen maakt TiP mogelijk minder vatbaar voor infectie dan niet geïmpregneerde allograft bot korrels [12-14].

Ten derde zou het fenomeen van galvanische corrosie kunnen optreden bij implantatie van een metalen implantaat. Hoewel dit onderwerp kort dient te worden, lijkt de bezorgdheid hieromtrent niet voor de hand liggend. Orthopedische implantaten die uit verschillende metalen bestaan en in direct of indirect contact staan met elkaar worden sinds vele jaren geimplanteerd. Tot dusverre is galvanische corrosie geen klinisch relevant item gebleken, zelfs bij plaatsing van de zeer poreuze Trabecular Metal® implantaten, welke een groot oppervlaker per gewicht hebben, vergelijkbaar met TiP. Bovendien is commercieel puur (cp) titanium een chemisch inert materiaal ten gevolge van snelle passieve oxidatie. Vanwege deze redenen is het onwaarschijnlijk dat implantatie van TiP in direct contact met, of in de nabijheid van, een ander metaal (bijvoorbeeld een cobalt chroom gaas of een cobalt chroom heup prothese) geassocieerd is met galvanische reakties van enige betekenis [15,16].

Een andere vraag verwijst naar één van de primaire doelen van defect reconstructie door middel van impaction grafting en betreft de toekomstige reviseerbaarheid van een reconstructieve laag van geïmpacteerde TiP. Een laag geïmpacteerde autograft of allograft bot korrels zal incorporeren en worden omgezet in nieuw eigen bot binnen één tot twee jaar na de operatie. Het is de vraag of een reconstructieve laag TiP, gewapend door ingegroeid bindweefsel en / of bot weefsel bewerkbaar is met conventionele chirurgische instrumenten zoals een reamer en een rasp. Van industriele toepassingen zoals de luchtvaart industrie is het bekend dat cp titanium (treksterkte 241 tot 550 MPa) beduidend makkelijker te bewerken is dan titanium legeringen (treksterkte tot wel 1380 MPa). Naast lage ream snelheden, welke gebruikelijk zijn in een chirurgische setting, zijn scherpe instrumenten en uitgebreid koelen met water absolute voorwaarden om te voorkomen dat het titanium wordt uitgesmeerd en aan de chirurgische instrumenten blijft ‘‘plakken’’.

Tot slot beschrijft dit proefschrift de toepassing van TiP met een *gecementeerde* techniek. Hoewel in de literatuur goede klinische resultaten zijn te vinden voor zowel

gecementeerde als ongecementeerde toepassing van acetabulaire bot impaction grafting, raden we af om impaction grafting van poreuze titanium korrels te combineren met een ongecementeerde cup [17,18]. Tijdens in vitro belasten van een TiP impaction grafting reconstructie (gecombineerd cavitair en segmentaal defect) met een ongecementeerde metal backed cup toonde de cup progressieve migratie. De hiermee gepaard gaande plastische deformatie van de TiP laag is wellicht het gevolg van een verhoogde stress in het oppervlak van de TiP laag waar deze in contact komt met de relatief stijve metal backed cup. Mechanisch gezien is dit een compleet andere situatie dan de geleidelijke afname in stijfheid bij een gecementeerde cup waarbij bot cement penetreert in het oppervlak van de TiP laag. De progressieve plastische deformatie van de TiP laag ten gevolge van belasten van de metal backed cup resulteerde in vroeg falen van de initieel press fit gefixeerde cup (niet gepubliceerde data). Het toevoegen van een cement laag tussen de harde metalen cup en de relatief zachte TiP laag van geimpacteerde TiP zou de druk krachten over een aanzienlijk groter gebied verdelen, beneden de faalgrens van de titanium transplantaat laag.

In overeenstemming met het advies om TiP niet als een volledig gewicht dragende reconstructieve laag rondom een niet gecementeerd kunstheup aan te brengen, zou het logisch zijn om een vergelijkbare strategie toe te passen voor de mechanisch zelfs zwakkere en meer vervormbare bot korrels. De goede klinische resultaten van ongecementeerde acetabulaire bot impaction grafting werden voornamelijk gezien bij cavitaire defecten [18]. In afwezigheid van een wezenlijk segmentaal defect zal een goed gefixeerde press fit cup de belasting van de onderliggende bot korrel laag verminderen vergeleken met een gecombineerd segmentaal en cavitair defect. Als gevolg zou de toepassing van TiP of bot korrels als vulmateriaal verdedigbaar kunnen zijn in ongecementeerde reconstructies van een klein cavitair defect.

Concluderend uit de verrichte onderzoeken voldoen calcium fosfaat gecoate TiP aan de primaire vereisten van een volledige bot vervanger voor gecementeerde acetabulaire impaction grafting. TiP zijn vervormbaar als bot korrels en bezitten derhalve gunstige eigenschappen om te hanteren tijdens een operatie. TiP kunnen geïmpacteerd en vervormd worden door de chirurg in overeenstemming met de betreffende (zeer variabele) geometrie van het bot defect en de afmeting van het geïmplanteerde kunstgewricht. Dit is een aanzienlijk voordeel vergeleken met andere poreuze metalen

vullers (voornamelijk Trabecular Metal®) die momenteel op de markt beschikbaar zijn: deze opvullers zijn voorgevormd, passen zich niet aan aan de geometrie van het defect en zijn niet verkrijgbaar als vervormbare korrels [11]. Na impactie creëeren TiP een macro poreuze laag die in staat is om direct postoperatief volledige belasting te weerstaan. Een laag geïmpacteerde TiP wordt binnen 15 weken na implantatie gewapend door ingroei van bot onder direct postoperatieve volledige belasting. Een zeer kleine hoeveelheid titanium partikels komt vrij tijdens de impactie van de TiP. Echter, na impactie waren er geen tekenen van titanium micro partikel vrijgave tijdens belasten. Er waren geen tekenen van versnelde slijtage van de polyethyleen cup. Hoewel de titanium bloed concentratie significant toenam, bleven de absolute waarden erg laag.

Aan het einde van dit proefschrift worden suggesties voor verder preklinisch onderzoek geformuleerd alsmede de omlijsting van een pilot studie bij mensen:
Om de osteoconductiviteit van TiP te optimaliseren zou de diameter van TiP minstens 2.8 – 4.0 mm moeten bedragen en zouden TiP gecoat moeten zijn met calcium fosfaat. Om de osteconductiviteit te behouden na (stevige) impactie, zou de gemeenschappelijke porie venster grootte tussen de geïmpacteerde korrels (inter-korrel poriën), en bij voorkeur ook binnen in de geïmpacteerde korrels (intra-korrel poriën) tenminste 100 µm dienen te bedragen. Verdere preklinische evaluatie van toepassing van TiP als een volledig bot vervangend materiaal voor gecementeerde *femorale* impaction grafting zou moeten bestaan uit een realistische belaste in vivo studie met reconstructies van een groot femur defect in een geschikt dier model (geit of schaap), vergelijkbaar met acetabulaire reconstructie. Aandacht zou moeten worden besteed aan het goed uitvoeren van de cementeringstechniek met vroege introductie van het cement en adequaat pressurizen om voldoende verstrengeling van het bot cement met het oppervlak van de geïmpacteerde titanium laag te garanderen.
Om over te gaan naar toepassing van deze titanium korrels op een grotere schaal, bevelen we aan om hieraan voorafgaand een prospectieve gerandomiseerde pilot studie bij mensen uit te voeren. Hierin zouden kleinere acetabulaire (AAOS type I en II) en femorale (Valle en Paprosky type I en II) defecten gereconstrueerd moeten worden door chirurgen die ervaring hebben met de impaction grafting techniek [19,20]. Deze pilot studie zal moeten uitwijzen of toepassing van TiP veilig is bij mensen op lange termijn

met betrekking tot de fractuur kans in osteopeen en osteoporotisch bot, mechanische stabiliteit van de reconstructie, vrij lichaam slijtage, wrijvingsslijtage en vermoeiing, afwezigheid van ongewenste weefsel reacties, stabilisering van titanium concentraties in bloed en reviseerbaarheid. Na succesvolle introductie van TiP in gecementeerde impaction grafting van kleinere defecten kan de toepassing uitgebreid worden naar een pilot studie met reconstructie van grotere defecten (AAOS type III en IV, Valle en Paprosky type III).

REFERENTIES

1. Li JP, Li SH, Van Blitterswijk CA, de Groot K. Cancellous bone from porous Ti6Al4V by multiple coating technique. J Mater Sci Mater Med 2006;17(2):179-85.
2. Bobyn JD, Pilliar RM, Cameron HU, Weatherly GC. The optimum pore size for the fixation of porous-surfaced metal implants by the ingrowth of bone. Clin Orthop Relat Res 1980(150):263-70.
3. Chang BS, Lee CK, Hong KS, Youn HJ, Ryu HS, Chung SS, Park KW. Osteoconduction at porous hydroxyapatite with various pore configurations. Biomaterials 2000;21(12):1291-8.
4. Itala AI, Ylanen HO, Ekholm C, Karlsson KH, Aro HT. Pore diameter of more than 100 microm is not requisite for bone ingrowth in rabbits. J Biomed Mater Res 2001;58(6):679-83.
5. Jones AC, Arns CH, Hutmacher DW, Milthorpe BK, Sheppard AP, Knackstedt MA. The correlation of pore morphology, interconnectivity and physical properties of 3D ceramic scaffolds with bone ingrowth. Biomaterials 2009;30(7):1440-51.
6. Welsh RP, Pilliar RM, Macnab I. Surgical implants. The role of surface porosity in fixation to bone and acrylic. J Bone Joint Surg Am 1971;53(5):963-77.
7. Basalah A, Shanjani Y, Esmaeili S, Toyserkani E. Characterizations of additive manufactured porous titanium implants. J Biomed Mater Res B Appl Biomater 2012;100B(7):1970-9.
8. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. Biomaterials 2005;26(30):6014-23.
9. De Man FH, Tigchelaar W, Marti RK, Van Noorden CJ, Van der Vis HM. 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.
10. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Matsushita T, Kokubo T, Nakamura T. Osteoinductive porous titanium implants: effect of sodium removal by dilute HCl treatment. Biomaterials 2006;27(13):2682-91.
11. Sporer SM, Della Valle C. Porous metal augments: big hopes for big holes. Orthopedics 2010;33(9):651.
12. Ketonis C, Barr S, Adams CS, Hickok NJ, Parvizi J. Bacterial colonization of bone allografts: establishment and effects of antibiotics. Clin Orthop Relat Res 2010;468(8):2113-21.
13. Radin S, Campbell JT, Ducheyne P, Cuckler JM. Calcium phosphate ceramic coatings as carriers of vancomycin. Biomaterials 1997;18(11):777-82.
14. Noda I, Miyaji F, Ando Y, Miyamoto H, Shimazaki T, Yonekura Y, Miyazaki M, Mawatari M, Hotokebuchi T. Development of novel thermal sprayed antibacterial coating and evaluation of release properties of silver ions. J Biomed Mater Res B Appl Biomater 2009;89(2):456-65.
15. Hol PJ, Molster A, Gjerdet NR. Should the galvanic combination of titanium and stainless steel surgical implants be avoided? Injury 2008;39(2):161-9.
16. Meyer H, Mueller T, Goldau G, Chamaon K, Ruetschi M, Lohmann CH. Corrosion at the cone/taper interface leads to failure of large-diameter metal-on-metal total hip arthroplasties. Clin Orthop Relat Res 2012;470(11):3101-8.
17. Wilson MJ, Whitehouse SL, Howell JR, Hubble MJ, Timperley AJ, Gie GA. The Results of Acetabular Impaction Grafting in 129 Primary Cemented Total Hip Arthroplasties. J Arthroplasty 2013.

18. Lee JM, Nam HT. 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.

19. D'Antonio JA, Capello WN, Borden LS, Bargar WL, Bierbaum BF, Boettcher WG, Steinberg ME, Stulberg SD, Wedge JH. Classification and management of acetabular abnormalities in total hip arthroplasty. Clin Orthop Relat Res 1989(243):126-37.

20. Valle CJ, Paprosky WG. Classification and an algorithmic approach to the reconstruction of femoral deficiency in revision total hip arthroplasty. J Bone Joint Surg Am 2003;85-A Suppl 4:1-6.

ACKNOWLEDGEMENTS (DANKWOORD)

Iedereen, genoemd en niet genoemd, die direct en indirect heeft bijgedragen aan het tot stand komen van dit boekje wil ik bedanken voor hun advies, vertrouwen, hulp, geduld en humor. Zoals niet ongebruikelijk in een dankwoord permiteer ik me enige ruimte voor nostalgie en sentiment.

Allereerst de heren van de manuscript commissie: Ide Heyligers, John Jansen en Harrie Weinans.

Chris, Pieter, Nico en Wim, jullie hebben mij erin geloodst en het benodigde vertrouwen gegeven. Dit heb ik altijd zeer gewaardeerd. Daarnaast kreeg ik alle ruimte om ''mijn ding'' te doen en te experimenteren, ook als voor jullie de rechtstreekse relevantie voor het lopende onderzoek ontbrak. De dataverzameling liep volgens plan. Echter de publicaties lieten op zich wachten. Confidentialiteit, patent rapport en een opleiding tot medisch specialist waren obstakels in het vaarwater. Uiteindelijk hebben onze inspanningen een interessant eindresultaat opgeleverd. Bedankt voor jullie geduld.

Léon en Willem, de goudhaantjes van het lab. Want al die woorden en ideeën moeten toch naar een tastbare testopstelling of bestudeerbaar materiaal vertaald worden. Altijd weer vrolijk, behulpzaam en adequaat.

Ineke, vlotte secretariële en morele ondersteuning met een aangenaam praatje erbij.

René, voor al je hand- en spandiensten. Ik heb veel aan je hulp gehad. Proficiat met je eigen PhD titel.

Gerjon, Dennis en Esther, dank voor jullie hulp, tips en overleg. En de vele opmerkingen over mijn niet altijd even gebruikelijke indeling van de dag. Wanneer ik weer halverwege de ochtend binnen kwam, hadden jullie al meerdere koppen koffie op en vele gigabytes muziek gedownload.

De mannen en vrouwen van het Dierenlab: Alex, Ton, Fred, Wilma, Jeroen, Conrad, Connie.

De afdeling Biomaterialen van de Radboud Universiteit Nijmegen. In het bijzonder de heren Sander, Joop en Jurgen.

De RD&E afdeling in de Maartenskliniek. Af en toe lekker bijbabbelen en in de avonduren wat SPSSen.

Wellicht onverwacht maar toch: de reviewers van de manuscripten. Hun kritische spiegel hielp één en ander uit te kristalliseren.

Jaco en Jorn. Medemusketiers en maatjes. Dank voor jullie luisterend oor en ontspannende (trainings)uren. Tijd voor een feestje!

Michiel, wij kunnen met elkaar over alles een goed gesprek voeren. Je bent een heel fijne vent.

Leon, miene broor. Ich haop nog vuhle uurkes saame door te bringe. Haopelik woone veer in de toekoms dichter biejein. Kleine William haet de allerfijnste suikeroom!

Mien elders. Pap en mam, bedank det geer mich altied miene eige gank leet gaon.

Arris, voor je meedenken en steun.

Dayenne. Bedankt voor al je steun, liefde, gezelligheid en geduld. Die kleine gaat jóuw promotietraject worden (knipoog).

William, jouw heerlijke lachjes, geluidjes en levensplezier hebben een ontwapenend effect op iedereen. Je bent altijd vrolijk en maakt me een heel blije en trotse papa. Ik kijk er naar uit om heel veel momenten samen met jou te beleven!

ABOUT THE AUTHOR

Luc Walschot (Lucas Hubert Bernard Walschot) was born on 10th October 1975 in Roermond, The Netherlands. He spent his youth in Herkenbosch, playing a lot with his brother Leon, Lego and radio controlled airplanes. Further, he played saxophone and clarinet in local wind orchestras and the Dutch National Youth Harmony Orchestra. In 1994 he graduated from high school (gymnasium) at the Bisschoppelijk College Schöndeln, Roermond.

After passing his first bachelor year in Medicine in 1995 at the University of Leuven, Belgium, he continued his Medicine study at the Radboud University Nijmegen, The Netherlands. After graduating as an MD and epidemiologist in 2002, he worked during one year as an orthopaedic junior house officer. From 2004 – 2006 he spent three years on performing research at the Orthopaedic Research Lab of the Radboud Universtiy Nijmegen Medical Centre (head: prof. P. Buma and prof. N. Verdonschot).

From 2007 – 2012 he was trained in the Netherlands to become an orthopaedic surgeon, starting with two years general general surgery at the VieCuri Medical Centre, Venlo (head: H.M.J. Janzing). Consecutively, four years of orthopaedic surgery took place at the Radboud Universtiy Nijmegen Medical Centre (head: A. van Kampen / M.C. de Waal Malefijt) and the St. Maartenskliniek, Nijmegen (head: A.B. Wymenga).

After his registration as an orthopaedic surgeon in December 2012 he did a fellowship in shoulder surgery at the Rijnland Hospital, Leiderdorp (head: C.P.J. Visser). From August 2013 – July 2014 he will be a fellow in trauma, foot and ankle and upper extremity at Flinders Medical Centre, Adelaide, Australia.

Dayenne Pronk and the author have a lovely son named William.

APPENDIX

COLOR FIGURES

CHAPTER	CORRESPONDING FIGURES
Chapter two:	1, 4
Chapter three:	1 – 3
Chapter four:	2, 5
Chapter five	1 – 3
Chapter six:	3
Chapter seven:	2 – 4

CHAPTER TWO

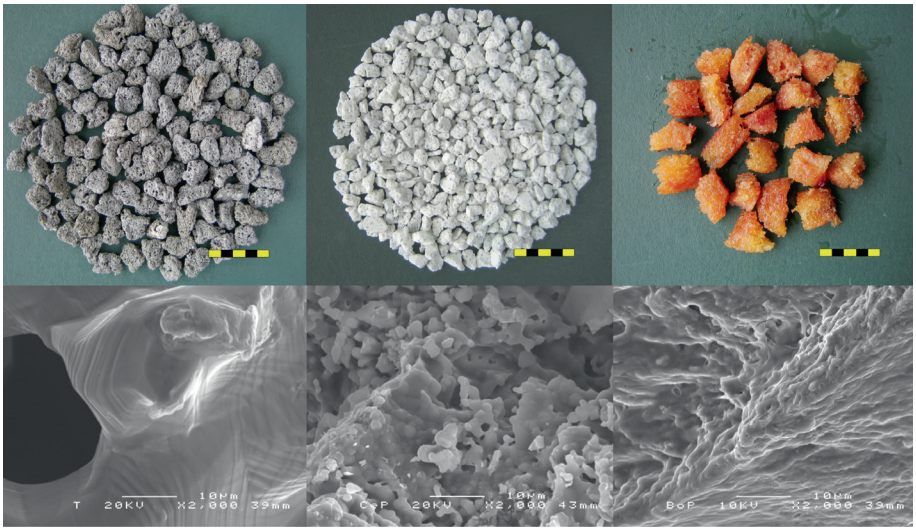


Figure 1 *Top left to right: TiP (3.0 g), CeP (4.0 g) and BoP (4.0 g), before impaction. The bar indicates 1 centimeter. Bottom left to right: close-up of the corresponding material surface (SEM, 2000x).*

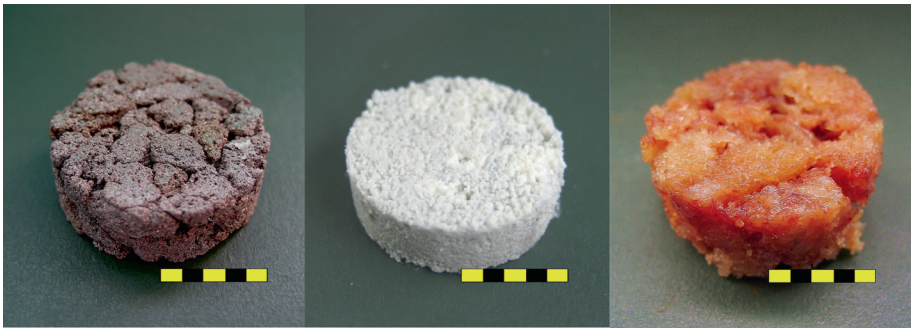


Figure 4 *Impacted specimens of TiP (firmly entangled), CeP (no entanglement) and BoP (some entanglement). The bar represents 1 centimeter.*

CHAPTER THREE

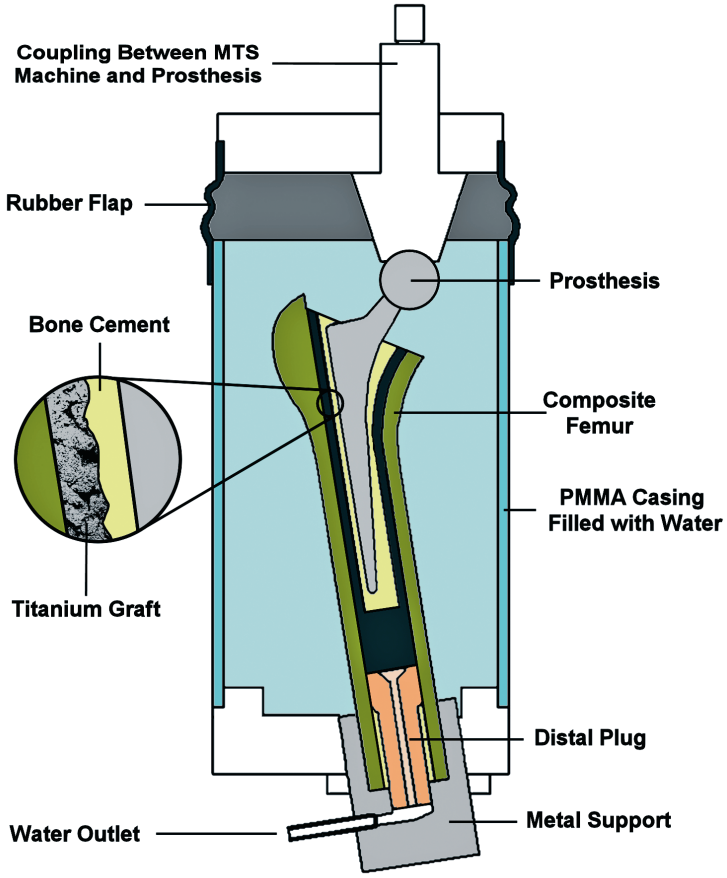


Figure 1 *A diagram provides an overview of the experimental setup. PMMA = polymethylmethacrylate.*

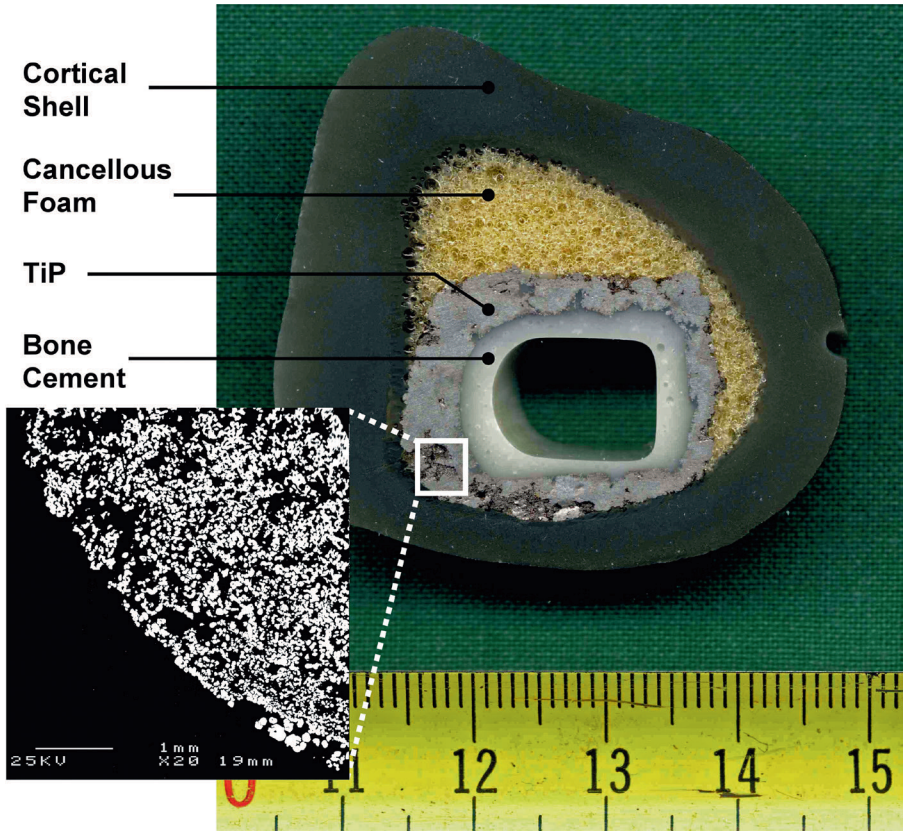


Figure 2 *A proximal transverse cross section with closeup shows a highly porous graft layer (backscatter SEM at 25 kV, 20x magnified). TIP = titanium particles.*

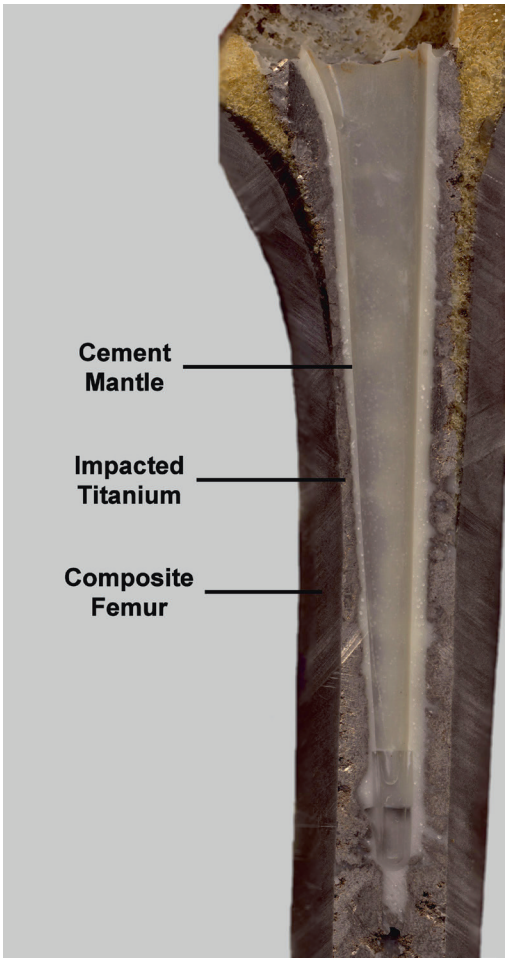


Figure 3 *A frontal section of one of the reconstructions shows the titanium graft layer, cement mantle, and cement penetration. The cement mantle on the medial side (left; 1.82 mm) is thinner ($p < 0.001$) than the cement mantle on the lateral side (right; 2.89 mm). The titanium graft is a porous structure but seems massive as an artifact of sectioning.*

CHAPTER FOUR

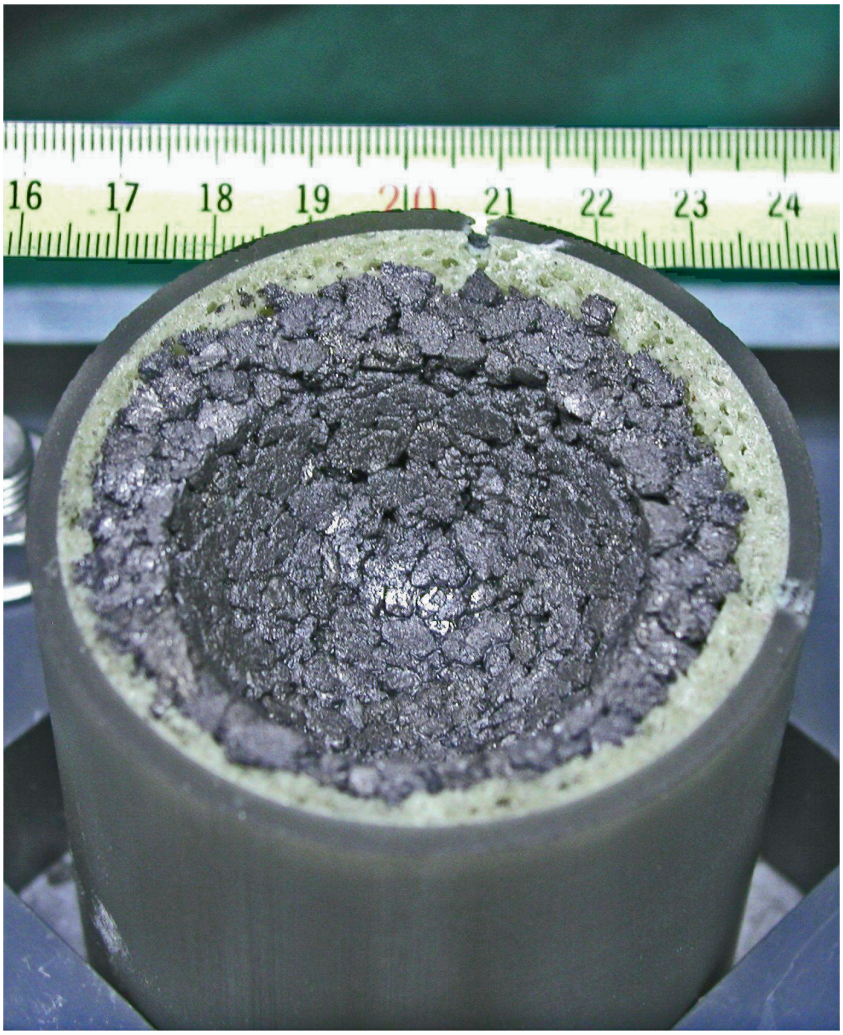


Figure 2 *A reconstructive graft layer consisting of porous titanium particles (T34). Note macroporosity and difference in supero-lateral and infero-medial graft layer thickness (10 mm vs. 4 mm).*

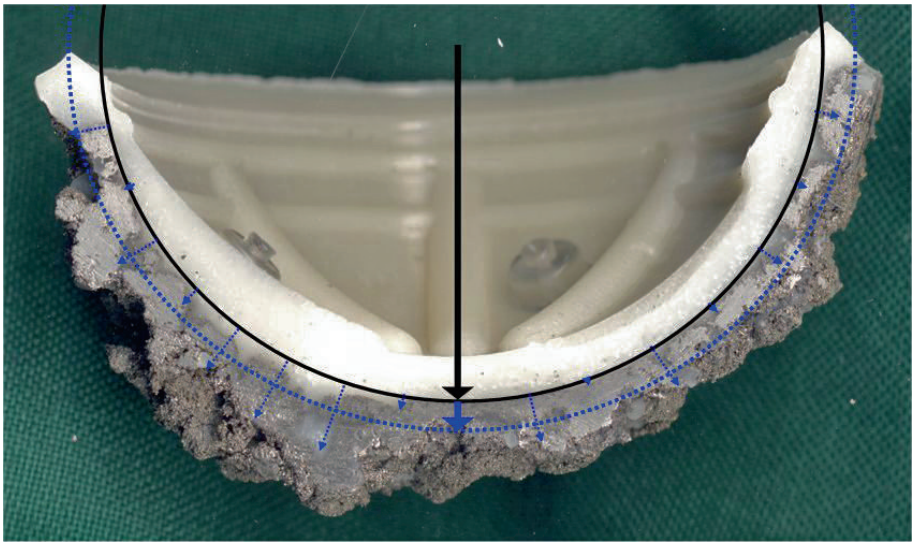


Figure 5 *Cross-section of a cemented cup with titanium particles after failure during the lever out test. The solid large arrow corresponds to the radius of the largest impactor (23 mm). Local variations in cement penetration depth are indicated by the dotted small arrows.*

CHAPTER FIVE

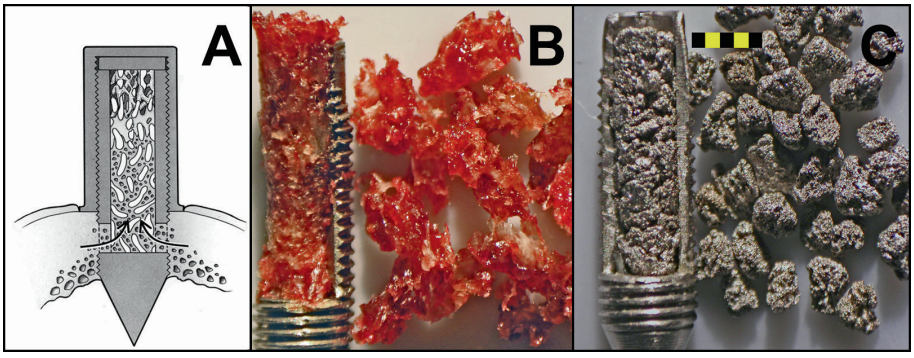


Figure 1 *A. Cross-sectional drawing of an implanted bone chamber in the proximal tibia of the goat. B. Cylinder of impacted bone particles in an opened bone chamber (left) with the corresponding amount of graft before impaction (right). C. Cylinder of impacted titanium particles (left) with the corresponding amount of graft before impaction (right). Scale bar represents 2 mm.*

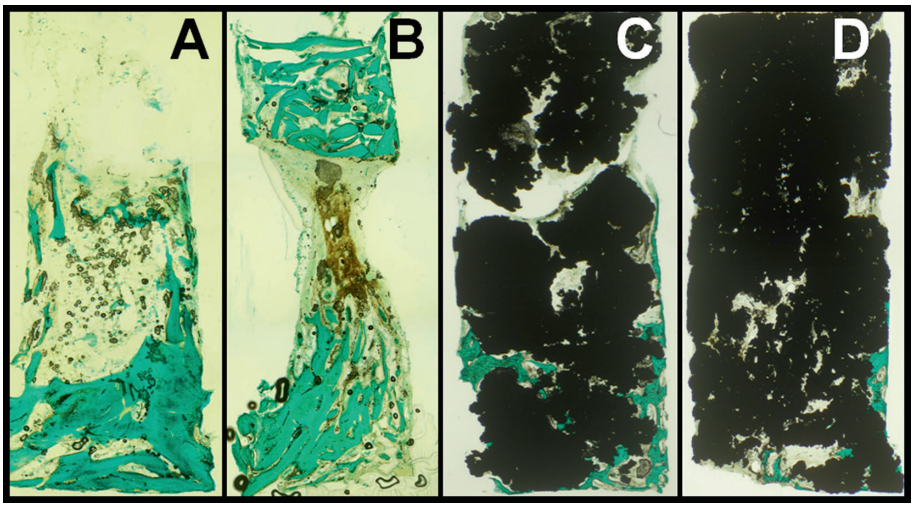


Figure 2 *Shape of the bone ingrowth front in empty chambers (A), allograft with graft remnants at the top (B), non impacted, coated titanium particles (C) and coated, impacted titanium particles (D (Goldner staining).*

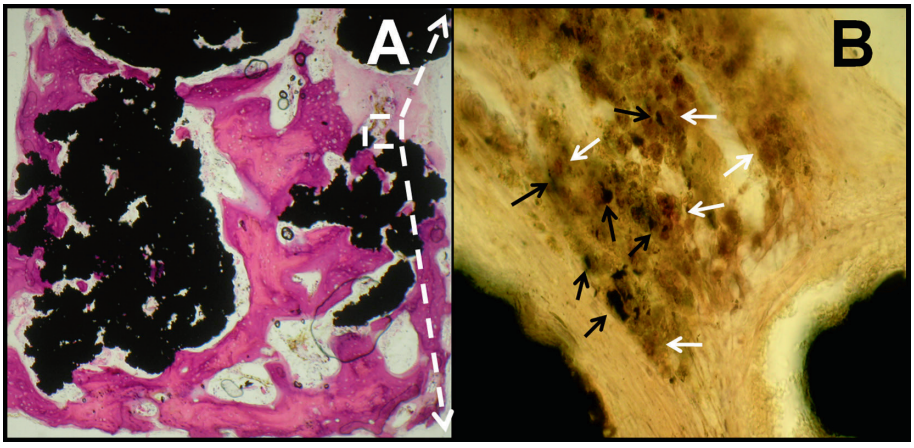


Figure 3 *A. Osteoconduction of titanium particles with bone-surface contact (HE staining). B. Acid phosphatase staining (400x) with titanium micro particles (black arrows) engulfed by macrophages (solid white arrows).*

CHAPTER SIX

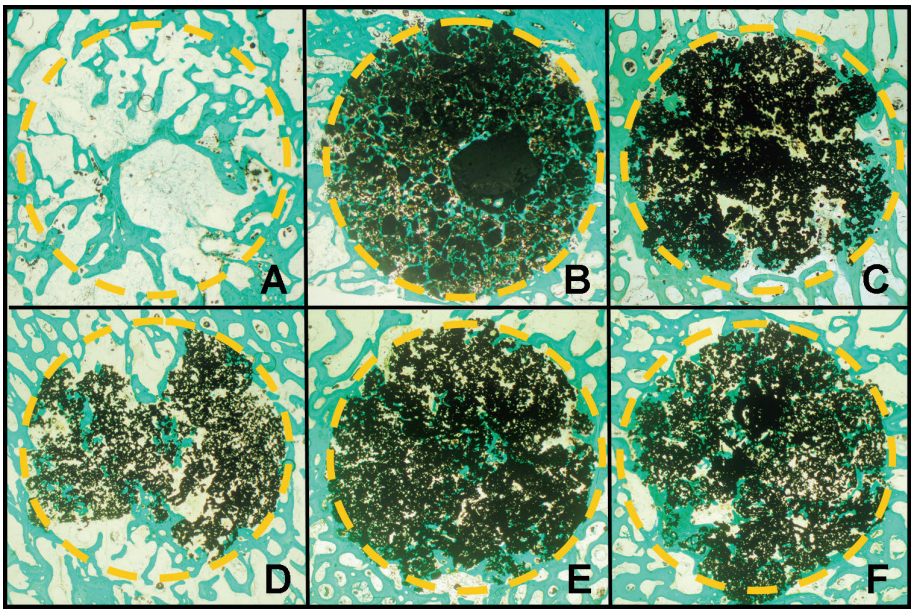


Figure 3 *Representative cross-sections 12 weeks after implantation, showing presence of bone (white) in the center of all graft groups except non coated TiP (A-F: B, CP, T, TB, TC1, TC2). The dotted circle outlines the original defect.*

CHAPTER SEVEN

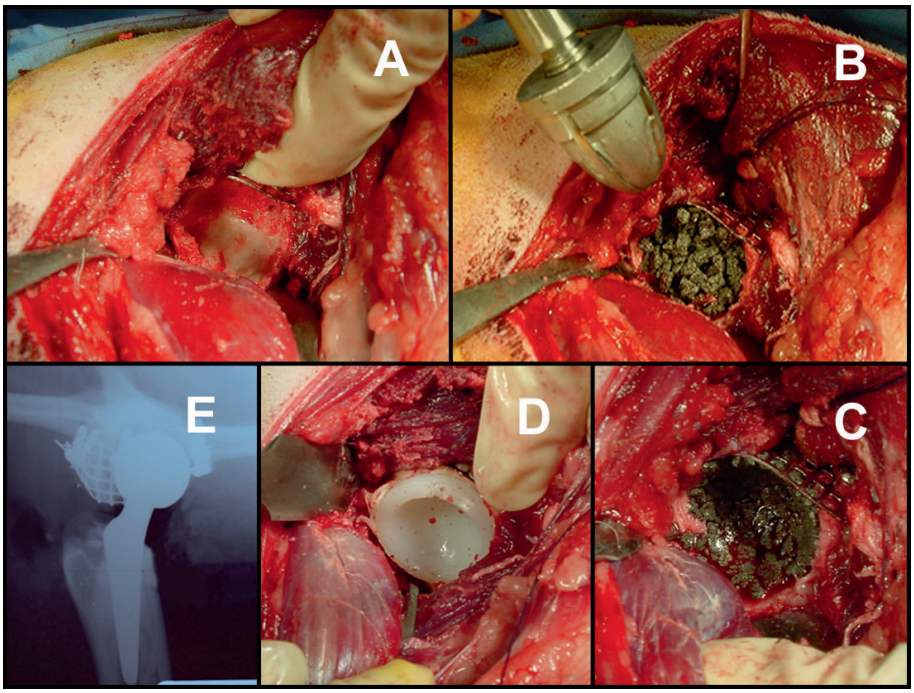


Figure 2 *A. An acetabular AAOS type III defect. B. Reconstruction with TiP. C. TiP graft layer after impaction grafting. D and E. Cementation of a downsized Exeter hip prosthesis.*

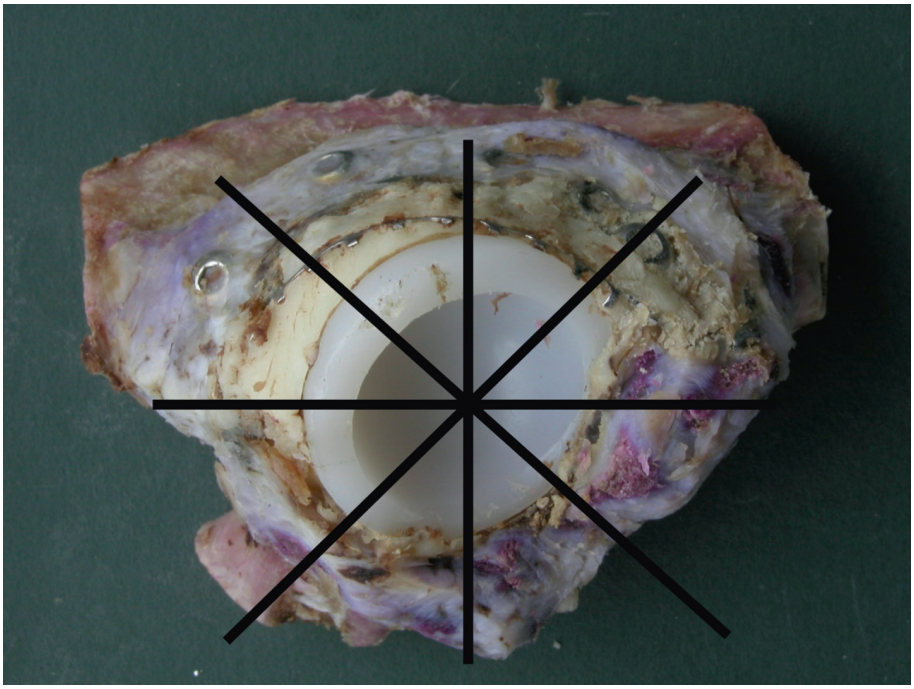


Figure 3 *Retrieved acetabular reconstruction15 weeks after implantation. The black lines indicate the orientation and location of the sections used for histomorphometry. Superolaterally, the rim of the metal mesh is visible as well as the heads of the screws used for initial fixation of the mesh.*

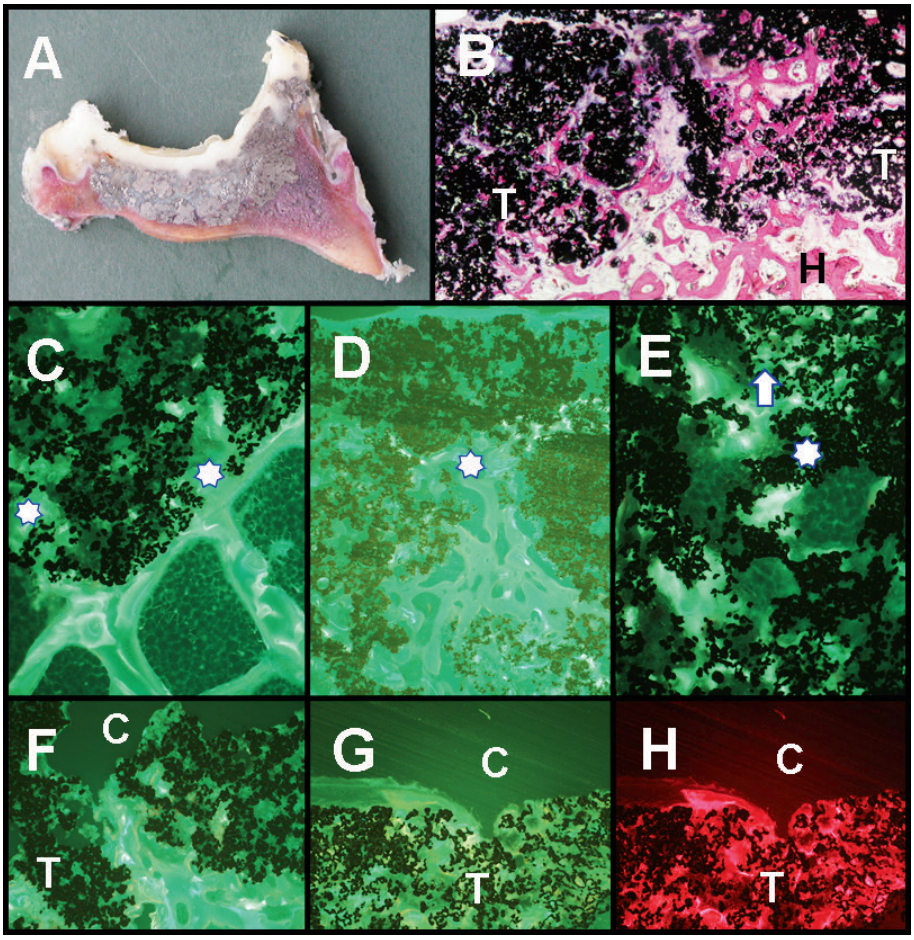


Figure 4 *A. Cross-section of reconstructed acetatubulum showing the host bone (H), TiP layer (T), and cement (C). B. HE stained detail of the host bone (H) TiP interface (T). C. Detail of host bone-TiP layer with calcein fluoresce. Asterisks indicate new bone. D. New bone formation in gap between two TiP particles (asterisks). E Calcein fluorescence indicating new bone formation in larger (asterisks) and smaller pores (white arrow) in TiP particle. F. TiP(T)-cement (C) interface. G and H Same section and location but with different filter sets showing calcein and tetracycline fluorescence (G) and alizarin fluorescence (H). Notice thin soft tissue interface between TiP (T) and Cement layer (C).*