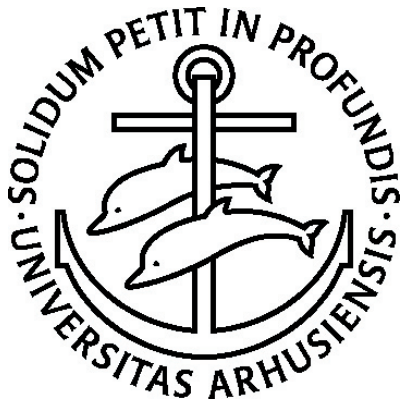
The influence of hydroxyapatite coating on the peri-implant migration of polyethylene particles

PhD thesis

Ole Rahbek



Faculty of Health Sciences
University of Aarhus
Denmark
2002

From the Orthopaedic Research Laboratory,
Department of Orthopaedics,
Institute of Experimental Clinical Research,
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List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-III).

I Rahbek O, Overgaard S, Lind M, Bendix K, Bünger C, Søballe K. **Sealing effect of hydroxyapatite coating on peri-implant migration of particles.** An experimental study in dogs. *J.Bone Joint Surg.Br.* 2001; 83:441-447

II Rahbek O, Overgaard S, Jensen TB, Bendix K, Søballe K. **Sealing effect of hydroxyapatite coating: a 12-month study in canines.** *Acta Orthop.Scand.* 2000; 71:563-573

III Rahbek O, Kold S, Bendix K, Overgaard S, Søballe K. **Superior sealing effect of hydroxyapatite coating compared with porous-coated implants.** Manuscript, 2002.

Study I was nominated for the New Investigator Award and partly presented at the 43rd Annual Meeting of Orthopaedic Research Society, San Francisco, USA, 1997. Paper II won the Acta Orthopaedica Scandinavica Award and was presented in part at the Nordic Orthopaedic Federation 50th Congress, Tampere, Finland, 2000 and at the Annual Autumn Meeting of Danish Orthopaedic Society 2000. The study was given the second price for best oral presentation by the Danish Orthopaedic Society.

Preface

This thesis is based on studies performed at Orthopaedic Research Laboratory, Department of Orthopaedics, Aarhus University Hospital during my employment as diploma student in 1995 (grant from the Danish Research Council) and during my employment as research fellow in 1999-2002 at the Department of Orthopaedics, Amtssygehuset, Aarhus University Hospital (grant from Aarhus University).
Surgical procedures and care of animals were performed at the facilities of Institute of Experimental Clinical Research, Aarhus University Hospital. Preparation of histological sections was done at Orthopaedic Research Laboratory and Department of Pathology, Amtssygehuset, Aarhus University Hospital.
The present studies were initiated by professor Kjeld Søballe, M.D., D.M.Sc. I was introduced to orthopaedic research by Kjeld in 1995, and I have valued his guidance highly since then. His contribution to the field of bone implant research is impressive. Likewise, my thanks go to Søren Overgaard M.D., D.M.Sc. for excellent supervision, constructive criticism and support. Furthermore, professor Cody Bünger M.D., D.M.Sc. is thanked for his support and valuable advises.
Associate professor Knud Bendix M.D., D.M.Sc. always took the time for discussions and I am grateful for his expertise in the evaluation of biopsies and bone-implant specimens.
The conditions for experimental orthopaedic research in Aarhus are outstanding. Professor Otto Sneppen, M.D., D.M.Sc. and professor Jens Christian Djurhuus, M.D., D.M.Sc are thanked for their great work in providing the good working conditions at the Orthopaedic Research Laboratory and Institute of Experimental Clinical Research respectively.
My co-workers and research fellows Søren Kold, M.D., Ulf Bromose, M.D., Thomas Bo Jensen, M.D., and Martin Lind M.D., D.M.Sc., are thanked for their friendship, good discussions, humour and help. I am grateful to Joan E Bechtold, PhD at the The Orthopaedic Biomechanics Laboratory, Hennepin County Medical Center, Minneapolis MN for her help and interest in my research.

Special thanks go to Anette Milton, Jane Pauli and Lis Lund for their knowledge and skills in the preparation of histological sections.
Analysis for endotoxins of PE particles was performed by Dr. Lennart Larsson, Ph.D., Department of Medical Microbiology, Dermatology and Infection, Lund University, Sweden and I am thankful for his valuable contribution to the studies. Statistical advices and constructive comments were given by associate professor Morten Frydenberg, Department of Biostatistics, University of Aarhus.

Above all, I wish to thank Charlotte Mikkelsen, M.A., mother of my dear son Hans, for critical reading of manuscripts, linguistic advices and a lot more…….

Acknowledgements

The studies in this thesis were supported by grants from the Danish Rheumatism Association, the Danish Health Science Research Foundation, the Institute of Experimental Clinical Research, University of Aarhus, Aahus University Research Foundation, the Consul J. Fogh Nielsen and E. Fogh Nielsens Foundation, Lykfeldts Legat, Illum Fondet, Grosserer Valdemar Foersom og hustru Thyra Foersom født Otto´s fond, Fonden til Lægevidenskabens Fremme and Søren Alfred Andersens Legat.
Biomet Inc. produced the experimental implants.

Contents

Abbreviations

CE	Coefficient of variation
HA	Hydroxyapatite
HA implant	Grit-blasted implant with HA coating
HA-po implant	Porous-surfaced implant with HA coating
HDPE	High density polyethylene
IL	Interleukine
IUR	Isotropic uniform random
MMA	Methylmetacrylate
MMP	Matrix metalloproteinase
PE	Polyethylene
PGE_2	Prostaglandin E2
PMMA	Polymethylmetacrylate
RSA	Roentgen stereophotogrammetric analysis
SD	Standard deviation
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
Ti-6Al-4V	Titanium-6aluminium-4vanadium
Ti implant	Implant with a grit-blasted titanium alloy surface
Ti-po implant	Implant with a plasma-sprayed titanium surface
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
TNFα	Tumor necrosis factor alfa
UHMWPE	Ultra high molecular weight polyethylene

Definitions

Aseptic loosening - Mechanical loosening of an endo-prosthesis without signs of infection.

Biomaterials - Material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body [163;164].

The effective joint space - Includes all peri-prosthetic regions that are accessible to joint fluid and thus accessible to particulate debris [130].

Gap healing - The tissue dimensions in the initial peri-implant gap evaluated by histomorphometry.

Histomorphometry - Quantitative evaluation of tissue dimension [110].

Implant - A medical device made from one or more biomaterials that is intentionally placed within the body, either totally or partially buried beneath an epithelial surface [163;164].

Ingrowth or ongrowth - The terms ingrowth and ongrowth were used for porous-coated and grit-blasted implants respectively. Bone in- or ongrowth was defined as direct contact between bone and implant surface at the light microscopic level [110].

Osteoconduction - A process that supports growth of different tissues involved in bone formation including vessels and osteoprogenitor cells from the host bone bed. Osteoconductive substances cannot induce bone formation at extraskeletal sites [47].

Sealing effect - The ability of an implant to reduce the effective joint space, thus reducing the access of joint fluid and wear debris to the bone-implant interface.

Stereology - A method based on observations made on two-dimensional sections, by which quantitative information is obtained about three-dimensional structures of objects [66].

Wear - Wear is defined by the removal of material from the prosthesis as opposed to creep, which is the plastic deformation of a material without the production of wear debris [97].

Abstract

Wear particles are believed to contribute to peri-implant osteolysis and to be a major cause of aseptic loosening of joint replacements. This PhD thesis addresses the clinical problems of wear debris migration at the bone-implant interface. The effect of wear debris in the peri-implant tissue, and methods to prevent access of the debris to the interface are studied. Furthermore, the possible dissemination of PE particles to distant organs is investigated.

The present thesis is based on 3 experimental animal studies of titanium alloy implants with and without HA coating. Study I examined grit-blasted implants at 16 weeks. The aim of the study was to investigate if HA coating could prevent the access of PE particles to the bone implant interface and hereby prevent any potential deleterious effects of PE particles. Study II examined the similar implants at 52 weeks to investigate whether the effect of HA found in study I was long-lasting. Furthermore, we examined whether inflammation and bone resorption in the bone-implant interface would occur due to chronic exposure of PE particles. Study III examined plasma-sprayed porous implants at 16 weeks. The hypothesis was that HA could further improve the sealing effect of a plasma-sprayed porous coating.

A modified stable weight-bearing Søballe implant device was used in all studies. Test implants were inserted in each distal femoral condyle of a dog and were surrounded by a 0.75 mm gap. The gap communicated with the joint space, allowing access of joint fluid to the bone-implant interface. PE particles used in the studies had a mean equivalent circle diameter of 2 (range 0.2-11) μm and were injected into the right knee joint three weeks after surgery. Injections were repeated at weekly intervals. The left knee joint served as control and received sham injections. Tissue specimens from the bone-implant interface were examined under plain and polarized light. The presence of PE particles along the interface, and the percentage of bone on- or ingrowth were quantified.

In study I, with grit-blasted surfaces, only few particles were found around HA-coated implants after eight weeks. In contrast, peri-implant tissue around Ti implants contained large amounts of particles. HA implants had approximately 35% bone ongrowth, whereas Ti implants had no bone ongrowth, and were surrounded by a fibrous membrane. PE particles did not cause inflammation or bone resorption in the peri-implant tissue.

In study II, also with grit blasted implant surfaces, injections of PE particles continued for a period of 49 weeks. HA implants had approximately 70 percent bone ongrowth and only few PE particles were found in the interface. No bone was seen on any Ti implants, all being surrounded by a fibrous membrane. Around Ti implants large numbers of PE particles were found in the bone-implant interface. The number of PE particles was evaluated semi-quantitatively. We found significantly fewer PE particles around HA implants compared to Ti implants with median values of 1 (0-1.5) and 4 (1.5-4) respectively ($p<0.002$). No significant difference in tissue-ingrowth was found between PE-exposed and vehicle-exposed implants. Infiltration of mononuclear inflammatory cells was present around 3 out of 7 Ti implants in relation to PE particles.

Specimens from iliac lymph nodes, liver, spleen and lung were examined. Dissemination of PE particles was only detected in regional inguinal lymph nodes.

In study III, with porous surfaced implants, PE particles were injected weekly until 16 weeks after surgery. The number of particles was reduced by the HA coating from a median value 2 (range 1-4) to 1 (0-1) ($p=0.01$). HA-po implants had approximately 60 percent bone ingrowth. Ti-po implants had virtually no bone ingrowth,

but were covered by a fibrous membrane. As in previous studies, no significant difference in tissue-ingrowth was found between PE-exposed and vehicle-exposed implants.

Conclusion

The present studies demonstrate that HA applied on a grit-blasted or porous surface yields superior bony ingrowth, and provides an initial protection against the migration of polyethylene particles in the bone-implant interface compared with non-HA-coated implants. After 52 weeks the sealing effect of HA remains. This long-term study indicates that inflammation due to PE particles in the bone-implant interface is a slow and time-dependent process, which may be inhibited by HA-coating.

Introduction

Clinical background

The first artificial joints were inserted, as early as in the last decades of the 19th century, however the results of these were disappointing. Through the 20th century experiments continued, but mechanical failures, fractures and infections were frequent complications. In the early sixties Sir John Charnley revolutionised orthopaedic surgery by the introduction of "low friction arthroplasty". Charnley introduced an artificial joint consisting of two components. The femoral component was made of metal and the acetabular was made of polyethylene. Both components were fixated to the surrounding bone by PMMA cement, which until then only had been used in dentistry. By introducing this concept, a good primary fixation of the implant and low friction between the articulating surfaces were gained. Simultaneously, the prophylaxis of infection was significantly improved and the number of deep infections was reduced.

Through decades, The Charnley prosthesis has been considered "the gold standard" and the basic principles in Charnleys design are still used today.

In Denmark, approximately 5000 primary hip arthroplasties and 900 revisions are performed yearly [94]. Primary osteoarthrosis (76 %), trauma (11%) and arthritis (6%) are the most frequent indications for operation [95]. One third of symptomatic osteoarthrosis in the hip or knee are treated with TKA or THA. Due to an increasing number of elderly people in the population the demand is expected to increase 30% the next 20 years [112].

Both THA and TKA are clinical successes. Patients are relieved for pain and maintain an acceptable level of physical activity. The overall risk for loosening and subsequently revision surgery is 5 % after 10 years [84;95]. However, the risk of implant loosening is much higher for younger patients.

Because of increasing numbers of THAs in younger patients, the demand for revision surgery has increased through the last decades and may likely proceed to do so in the years to come [68;95]. In general, the major cause for revision surgery is aseptic loosening (76%). Clinical studies strongly indicate a relationship between wear debris and aseptic loosening. The prevalence of osteolysis around the femoral component rises significantly with increasing wear of the acetabular component [138] and the number of polyethylene particles in peri-implant tissue is significantly higher in areas with osteolysis [85].

Cemented technique is considered standard treatment for elderly patients with low activity level and short life expectancy. For younger patients the cementless technique has been used because of the unsatisfactory results for this group with cemented technique. Cementless fixation relies on primary mechanical fixation of the implant followed by bony ingrowth into a textured or porous implant surface. Early reports on uncemented components were disappointing, but a resent report from the Norwegian hip register shows that since the uncoated uncemented designs were abandoned in the early 1990´s, there has been a clear improvement in the overall results of the uncemented implants. In fact, circumferential HA-coated stems have better survival rates with 0-11 years follow-up than cemented and porous-coated stems in patients younger than 60 years of age. However, the HA-coated cups still have inferior results compared to the cemented Charnley cup due to aseptic loosening [68].

Biomaterials

Titanium alloy

Implants used in the current studies are titanium alloy implants (Ti-6Al-4V). Other materials used for orthopaedic implants are cobalt chrome, stainless steel and commercial pure titanium. Titanium alloy is often used for non-cemented implants, because the elastic modulus is closer to that of cortical bone. This may reduce the stress shielding around the implant. Furthermore, titanium has been shown to be highly biocompatible.

Surface properties

On commercially available implants a variety of surface textures can be found. The surface can either be polished or roughened by various techniques such as grit-blasting, etching or by coating. Coated implants are implants with an additional layer of material added on the surface. This layer will typically consist of a porous metal structure and/or a calcium phosphate layer.

Porous coated implants

The porous surfaced implants are all characterized by channels called pores. The idea is that the bone grows into the pores and hereby the implant is osseo-integrated. Optimal pore-size for bone ingrowth has been determined to be between 50 and 400 μm [23].

Different kinds of porous coating techniques exist. By plasma-spraying a heated metal powder is sprayed onto the implant surface. Hereby a closed-pore surface is created. Open-pore coatings are produced by other techniques. A beaded surface is created by sintering technique. The implant is heated to high temperature (1200-1320 °C) so beads can be bonded to the surface. The heating process results in 50 percent decrease in the fatigue strength of Ti and therefore beaded surfaces are only applied on Co-Cr.

Fibermesh coating is created by a lower temperature (760-980 °C) by a process called diffusion bonding. Ti wires are moulded under compression onto the implant surface.

Hydroxyapatite

HA is one of several ceramics used for orthopaedic implants. HA is a natural occurring and the most prevalent mineral in bone tissue. The chemical formula of HA powder is: $CA_{10}(PO_4)_6(OH)_2$. If HA is heated to temperatures above 1300 °C, increasing amounts of HA is converted to tricalcium phosphate, thus lowering the crystallinity of the material. A HA coating with low crystallinity is more rapidly resorbed in bone tissue [111], because low crystalline coating releases more calcium and phosphate ions due to dissolution. This release enhances bone formation, thus giving the coating a higher bioactivity [42;65;92;96].

Experimental background

In 1987, de Groot and others published results of plasma sprayed HA-coated bone implants [38;43;56;79]. It was demonstrated that HA has osteoconductive properties and that the mechanical fixation of HA-coated implants is better than uncoated implants during optimal surgical conditions (press fit).

Extensive experimental studies in dogs conducted by Søballe et al [141;142;145;148] have proven that HA modifies tissue ingrowth into a porous surface during both stable and unstable loading conditions. Micromotion between bone and a Ti implant prevented bony ingrowth and resulted in development of a fibrous membrane. HA coating induced a membrane with presence of fibrocartilage, higher collagen concentration, radiating orientation of collagen fibers, and a thinner membrane as compared with Ti implants. The membrane around HA-coated implants was gradually converted to bone, whereas the membrane around Ti implants persisted.

Other studies by Søballe et al demonstrated that HA had the ability to brigde a peri-implant gap by bi-directional bone growth both with and without the presence of bone allograft in the gap [144;146].

Overgaard et al [113;116] investigated the importance of the underlying surface texture onto which HA is applied. Macroscopic evaluation of the implant

surface after push-out testing revealed that grit-blasted implants had pronounced delamination of the HA coating in contrast to porous-coated implants indicating that the bonding strength of HA on porous-coated implants was greater. Grit-blasted implants had greater bone ingrowth compared with porous-coated implants indicating different surface activities on the implants.

Clinical background

Clinical experience with HA coating is increasing, but there is still a need for long-term follow-up studies. However, numerous short-term studies have shown promising results [28;50;53;55;57;78;80;87;103;107;108;123;149;152]. Studies using roentgen stereophotogrammetric analysis (RSA) have demonstrated that HA coating is capable of reducing the early migration of both femoral hip and tibial knee components [78;80;103;107;108;149]. In addition, human retrievals have documented good bone apposition suggesting stability between implant and bone [13;14;37;143;153]. Coathup et al [36] compared ingrowth of bone to 21 stems of similar designs retrieved post mortem, matched according to their length of time *in vivo,* and found superior ingrowth to porous HA surfaced implants compared to plain porous and interlock implants.

Studies on HA-coated femural stems and acetabular cups are summarized in table 1. None of the studies have shown any adverse effect of HA coating. It has been proposed in case reports that HA particles originating from the implant coating could induce increased wear of the polyethylene liner due to third body wear [20;21]. However, no large clinical studies have reported increased wear by the use of HA [45;107;151;170]. Randomized prospective studies using RSA must be considered the gold standard comparing fixation of different surface coatings to bone. Three of such studies exist on the femural stem comparing proximal porous coating with or without HA [35;77;149]. Two of the studies demonstrate less migration of HA-coated implants, whereas one study shows equal migration with or without HA. The longest follow up on HA-coated stems is 10 years showing excellent results for the femural stem with a survival on 99% [98]. Follow-up after 8 and 10 years have shown comparable good results [54;122]. A striking finding in these series is the lack of osteolysis. Some calcar resorption is found but distal osteolysis is almost never seen.

Polyethylene

Polyethylene as bearing surface was introduced by Sir John Charnley to ensure low friction against the metal femoral head in the artificial joint.

Since then, the most widely used configuration has been a metal component articulating against a polymeric component, although other bearing couples exist such as metal-on-metal, metal-on-ceramic and ceramic-on-ceramic. Worldwide, approximately one million UHMWPE components are implanted per year [88].

Although, low friction arthroplasty has been a success, wear of the implanted components has been a matter of great concern. Many factors, such as type of polyethylene, additives, processing method, sterilisation method, packaging, cross-linking and design of the component, influence the wear of polyethylene. These factors must be considered in the production of polyethylene acetabular components.

Material properties

Polyethylene is a polymer consisting of long CH_2-chains. The polymer consists of crystalline lamellae embedded in non-crystalline amorphous regions. The polyethylene used for orthopaedic implants is UHMWPE. UHMWPE is defined by ASTM (American Society for Testing and Materials) D 4020 as a linear polyethylene with an average molecular weight of greater than 3.1 million g/mol. ISO (International Standards Organization) 11542 designates a linear polyethylene with molecular weight higher than 1 million g/mol. When Charnley applied polyethylene for surgical use, he called it High Density Polyethylene (HDPE), by which we today understand polyethylene with a linear polyethylene with a molecular weight below

Table 1. *Review of studies concerning HA-coated THRs.*
Design of the studies numbered as follows: Randomized prospective = 1, Matched pair = 2, Historical controls = 3, Follow-up without control = 4;
Method; a: RSA, b: conventional X-ray.
#: paired design, ¤: 32 mm heads, gamma sterilisation in air.

Authors	*Design/ Method*	*Implant*	*Time in situ*	*Polyethylene wear*	*Osteolysis*	*Migration*	*Survial*
Søballe et al 1993 [149]	1 a	Stem: Proximal porous coating +/- HA (Bimetric)	1 year	Not reported	Calcar resorption in both Ti (25%) and HA (21%)	Less migration of HA-coated stems	100 % in both groups
Ciccotti et al 1994 [35]	1 a	Proximal porous coated stem. HA-augmented vs non-HA (Taperloc)	2 years	Not reported	Not reported	No difference	100 % in both groups
Karrholm et al 1998 [77]	1 a	Cemented vs proximal porous-coated pad stems vs proximal HA (Tifit)	5 years	Not reported	Porous stems had more radiolucent lines	Less migration of HA as compared to cemented	Cemented 95% Porous 95% HA 100%
Dorr et al 1998 [45]	1 b #	Stem: Proximal porous coating +/- HA (Porous anatomic replacement stem I and II)	Mean 6.5 years	No differences	Fewer radiolucent lines around HA in proximal zones	No difference	100 % in both groups
Yee et al 1999 [170]	1 b	Stem: Proximal porous coating +/- HA (Mallory-Head)	Mean 4.6 (3-7) years	No differences	No difference	No difference	100 % in both groups
Thanner et al 1999 [151]	2 a	Fiber mesh porous cup +/- HA/TCP (Harris-Galante cup)	2 years	No differences	Fewer radiolucent lines around HA	Less migration for HA/TCP	100 % in both groups
Rothman at al 1996 [124]	2 b	Omnifit stem: Proximal porous coating +/- HA	Mean 2.2 years	Not reported	No differences	No differences	100 % in both groups
Onsten et al 1996 [107]	3 a	Omnifit HA-coated cup and stem (proximal 1/3) vs cemented Charnley	2 years	No differences	Stem: no osteolysis Cup: radiolucent lines around 10 %	No differences between groups	100% for stem and cup
Kroon et al 1992 [87]	3 b	Ti alloy ridged press fit stem vs proximal 1/4 HA	1 year	Not reported	No proximal radiolucent lines around HA-stems	Less migration of HA-coated stems	100 % in both groups
Geesink et al 1997 [54]	4 b	Omnifit stem with 1/3 HA coating and HA-cup	Mean 8 years	22 % of PE liners with head eccentricity, mean wear 1.63 mm	No distal osteolysis. Calcar resorption in 80 % of cases.	Not reported	Stem: 98 % Cup: 95%
Rokkum et al 1999 [122]	4 b	Landos Corail fully HA coated screw cup and stem	7-9 years	26 % PE liners revised or planned to be revised ¤	No distal osteolysis. 67% showed periarticular osteolysis	Not reported	Stem: 99% Backing: 93% PE inlay: 78 %
McNally et al 2000 [98]	4 b	Furlong fully HA-coated stem	Mean 10 years	Not reported	No distal or proximal osteolysis. Only benign calcar resorption	Not reported	Stem: 99%
Patients younger than 50 years							
Loupasis et al 1998 [93]	4 b	Furlong fully HA-coated stem	Mean 6 years	No definite evidence of wear was detected	Distal osteolysis in 2 %. Focal calcar progressive ostelysis 57 %	No migration of stems	Stem: 93%, none revised for aseptic loosening
Capello et al 1997 [29]	4 b	Omnifit stem with 1/3 HA coating	Mean 6.4 (5-8.3)	Not reported	No distal osteolysis. Proximal errosive scalloping in 32 %. No distal osteolysis	Not reported	Stem: 97.4%, none revised for aseptic loosening

200000 g/mol (ASTM D 4020). It is believed that the polyethylene used by Charnley by the current standards is UHMWPE and that lower molecular weight polyethylene never has been used for clinical purpose. There has simply been a change in nomenclature [88].
Still, UHMWPE has undergone large changes since it was introduced as a bearing material.

Additives
Some types of UHMWPE are added calcium stearate as catalyst for UHMWPE production. Furthermore, calcium stearate also acts as lubricant and a release agent in the process. However, trace elements of calcium stearate in UHMWPE have been associated with fusion defects and oxidation of the polyethylene. This has important influence on the ultimate tensile shear strength and fatigue resistance [88].
The UHMWPEs on the market today are mostly with calcium stearate, but manufactures are now switching to UHMWPE resins with lower levels of calcium stereate.

Processing of polyethylene
Polyethylene is produced as a powder (resin). Therefore, it is necessary to consolidate the resin before implant components can be machined. Optimal consolidation is crucial for good clinical performance of the polyethylene. Consolidation is done in two different ways: ram extrusion and compression molding [17]. Important parameters in both fabricating methods are time, temperature and pressure. They influence the density, crystallinity and degree of consolidation of polyethylene. When polyethylene is fabricated, the resin is melted and the crystallinity of the material decreases to approximately 45-55 percent. It is important that the crystallinity is restored when the material is cooled. If the material is not cooled at the proper rate, optimal crystallinity is compromised.

Sterilisation method and cross-linking
Until 1995, UHMWPE was typically sterilised in an oxygen environment using 25 to 45 kGY. However, this practice was changed, when it was discovered that the accelerated oxidation of UHMWPE, which was a consequence of this procedure, led to increasing wear rates. By irradiation of the C-chains, free radicals were induced. Free radicals combine with oxygen causing oxidative chain scission. The result is a polyethylene with less desirable wear properties. If oxygen is not present, the reaction cannot occur and cross-linking between the CH_2-chains occurs. Cross-linking of polyethylene highly reduces the wear of the material [88;109].
The companies are now sterilising the polyethylene in different ways to avoid the problems with oxidative chain scission and some companies use cross-linking to improve their products.
After the components are produced and sterilised, they must be stored properly to avoid oxidative degeneration during shelf storage.

Wear of polyethylene
Wear is not only linked to the quality of the polyethylene, but also the size of the femoral head [70] , and the thickness of the PE liner [91].
The wear mechanisms can be divided into 4 wear modes. Mode 1 is adhesive wear between the two articulating bearing surfaces. Mode 2 refers to the condition of a primary bearing surface rubbing against a secondary surface in a manner not intended by the designer. This is typically seen when the head has worn through a polyethylene insert. Mode 3 is also called third body wear. This type of wear occurs when particulate material is interposed between the bearing surfaces and the surfaces become abraded. Mode 4 refers to two secondary surfaces rubbing together. This could be the fretting between a metal shell and a polyethylene insert [97].
Wear debris extracted from peri-implant tissue of failed implants has been analysed by SEM. Around cementless total hip replacements the mean size of PE particles has been determined to 0.5 μm ranging from 0.2 – 2.0 μm based on a frequency distribution. Most particles were found to be spheroids, however fibrils typical 0.2-0.3 μm broad and up until 10 μm in length were also found. Many particles were aggregated as a carpet

like mesh of 50 to 80 μm [136]. Particles of metal and other implant materials were also found. These findings of mainly submicron particles in peri-implant tissue have been confirmed in later studies [27;72;97].

The volumetric wear of an 8 mm thick polyethylene liner in cementless metal-backed implants (28 mm head) has been measured as high as 155.1 cubic millimetres per year in patients with a mean age of 64 [44]. These patients typically have a walking activity of approximately 5,000 steps per day [132]. If we assume that all wear particles produced, have a diameter of 0.5 μm and no creep is present, we can calculate the number of particles produced on a yearly basis to 2.4 x 10^{12} particles. Number of particles produced per step can thus be calculated to approximately 1 million particles per step.

Migration of wear debris

It has been recognised that distal osteolysis around femoral stems with circumferential proximal coating is a rare finding as compared with stems with no coating or with non-circumferential coatings. These findings could be due to a sealing effect of the interface around coated stems. Such an effect has been suggested for the plasma-sprayed [48], beaded [22] and HA-coated implants [54].

Wear debris is not only found in the peri-prosthetic tissues, but are also transported to lymph nodes and distant organs. Multiple polyethylene particles have been demonstrated in regional and pelvic lymph-nodes and traces levels of metal have been detected in bone marrow, liver and spleen [31;154].

Aseptic loosening

Histopathological findings

In 1977, Willert described [165] the synovial thickening and scar tissue around artificial joints. Huge amounts of wear debris were found in the articular capsule and the particles initiated a granulation tissue, including macrophages and foreign body giant cells. It was suggested that in cases, where the wear products from the artificial joint were not sufficiently removed by the lymph, the membrane could extend to the bone-implant interface and contribute to implant loosening.
Since the findings of Willert, research has mainly focused on the interfacial tissue. However, it is important to note that the total mass of biological active cells remains relatively small in the interfacial membrane compared with the pseudocapsule, where the lining is rich in macrophages, lymphocytes and fibroblast-like cells [106]. Levels are elevated of MMPs, TNFα and IL-1 in the joint fluid from artificial hip joints [104] and osteolysis may be due to the penetration of joint fluid to the interface.

Interfacial membranes

The interfacial membrane surrounding loose THRs has the histological characteristics of a synovial membrane [60]. Against the cement a single layer of large polygonal cells with eccentric nuclei is found. Beneath this layer a fibrovascular stroma is found with the presence of wear debris, macrophages and foreign body giant cells. The membrane produces high levels of osteolytic mediators [4;61;169].

Localised lysis

The peri-implant areas accessible to joint fluid and thus to wear debris have been termed " the effective joint space" [130]. Cracks in the cement mantle may allow access of particles to the interface without the presence of an interfacial membrane [8]. Via the space between the stem and cement, such defects provide a route through which the contents of the joint cavity may reach the endosteal surface of the femur, subsequently leading to localised bone lysis. The localised bone loss may be a distinct entity different from the interfacial membrane also termed linear osteolysis [125;126;166]. The aggressive granulomas in the localised bone loss consist of well-organized connective tissue containing macrophages and fibroblastic reactive zones. The granulomas are highly vascularized, and villous structures are observed inside the granuloma.

Macrophage response to wear debris

The particulate debris is phagocytosed mainly by macrophages. Particles within the range of 0.2-10 μm can be fagocytosed, however, particles found inside macrophages are mostly submicron [18]. Wear debris may consist of different materials used for prosthetics devices, such as polyethylene, metal, polymethylmethacrylate and ceramics. The response on the wear particles includes a large variety of cytokines, chemokines, arachedonic acid metabolites and degradative enzymes all interacting in a complicated network [4;33;51;58;61;81;101;102;105;169]. Cells of the monocyte/macrophages are recruited and some are differentiated in to osteoclasts by the activation of the RANK receptor RANKL [34]. Activated macrophages may even directly participate in bone lysis around the implant [74]. Among the most important cytokines in osteolysis are TNF α and IL-1 which both directly affects the osteoclast and osteoblast resulting in bone resorption. Both cytokines may also induce secondary effects on other cells resulting in the release of matrix degrading enzymes such as the different subtypes of human matrix metalloproteinases [73;117].
Some researchers have indicated that macrophage-mediated peri-implant osteolysis may not be the only process involved in aseptic loosening. The systemic cellular immune response may also be involved at least for predisposed individuals [74;167;168]

Factors, which may affect the response on wear debris, are listed in table 2.

Table 2

Factors affecting the response on wear debris	
Particle-related	Concentration [25] Size [62;64] Shape / surface area [58;62;134] Surface charge / chemistry [75;150] Composition [134;135] Endotoxin contamination [3;9;19;41;119]
Host-related	Predisposition [32]
Mechanical	Micromotion [10;16;49]

Endotoxins

Resent research has focused on the level of endotoxin on wear debris, since presence of endotoxins may explain why some experimental studies find osteolytic effects of particulate material and some do not (see discussion). Endotoxins are a common name for components (lipopolysaccharides) in the bacterial wall of Gram-negative bacteria that induce the inflammatory response by the host. Endotoxins are able to provoke a high inflammatory response by activating both the humeral and cellular immune system. Furthermore, endotoxins stimulate TNFα–dependent osteoclast differentiation and bone resorption [1;7;19].

A common method for detecting endotoxins on particles has been to measure the endotoxin level using the Limulus analysis on supernatants obtained by centrifugation of particle suspensions. However, only 1 percent of the endotoxins adherent to the particle surface is detected by this method [119].

A newly developed method using gas chromatography/tandem mass spectrometry [140] has enabled the accurate determination of endotoxin even when present down to trace levels in chemical complex matrices. Before analysis, the particles are hydrolysed and hereafter the sample is analysed for endotoxin specific free fatty acids by gas chromatography / tandem mass spectrometry. This method having a very high specificity and sensitivity, represents a chemical-analytical alternative to Limulus test.

Methodological considerations

Experimental animals

Previous studies concerning bone–implant research in relation to wear debris-mediated osteolysis have been conducted in several different species. The most commonly used animals are rats, rabbits, sheep and dogs.

The dog was chosen for our studies for a number of reasons. Our implant model requires an animal of a certain size and dogs weighing more than twenty kilos have appropriate sized femoral condyles. The individual variation is low, since the dogs are inbreed and originate from the same litters. Furthermore, dogs are easy to handle and adapt quickly to the experimental procedures such as injections.

The qualities of dog bone are quite similar to those of human bone. By comparing the composition of the trabecular bone, bone mass and mechanical bone properties between species of larger animals (humans, dogs, pigs, cows and sheep) it has been found that the characteristics of human bone are best approximated by the properties of dog bone [2]. The remodelling rate of dog bone is approximately 2-3-fold higher than in humans [83].

A study by Spector et al has compared interfacial tissue from loose femoral stems from dogs and humans [139]. It was demonstrated that dogs develop a synovium, similar to that observed around loose human prostheses. An equal secretion of osteoclast activating cytokines was also found in dog and human peri-implant tissues.

Another important reason to use the dog is the long history of research in this animal at our institution. Dogs have been used in studies concerning the biocompatibility of implant surfaces and graft materials in normal and osteopenic bone. Furthermore, a substantial number of studies concerning growth factors and new surgical techniques have been conducted in the dog.

Ethical considerations

Animal studies were all approved by the Danish Control Board for Animal Research. The dogs were bred for scientific purposes and treated in compliance with Danish laws for the use of experimental animals.

Design of the studies

In our studies, several hypotheses are tested. The strongest design was used for the hypothesis of highest priority. In all studies, the main topic was to investigate the effect of the PE particles on the bone implant interface. Therefore, a paired design was chosen with the contra-lateral knee serving as control (Figure 1). Two different types of implant coatings were investigated in each study. An implant was inserted into each femoral condyle in order to reduce variance between individuals and to minimise the number of animals needed. Implants of one kind were randomly allocated to lateral or medial condyle to compensate for differences in bone density, flow of joint fluid and loading conditions between sites of implantation.

The comparison between migration of PE particles in the interfacial tissue surrounding different implant surfaces was studied in an unpaired design. Injections of PE particles or control solution were conducted in both lateral and medial joint chamber to ensure an equal distribution of the injected material. PE particles were consequently injected into the right knee joint in order to simplify the procedure assuming no difference between right and left knees. An eventual difference between left and right side could have been eliminated by randomisation.

Particle dose was based on an estimated weekly production of 9.6×10^9 particles in a human total hip arthroplasty [97]. Assuming, that the effective joint space in a human hip has a volume of approximately 45 mL, the weekly load was calculated to be 2×10^8 particles per mL. By injecting 6×10^9 particles per week in the knee joint of a dog (assumed volume: 15 mL), we exposed the joint cavity with a twice as high load as compared to the human hip, namely 4 x

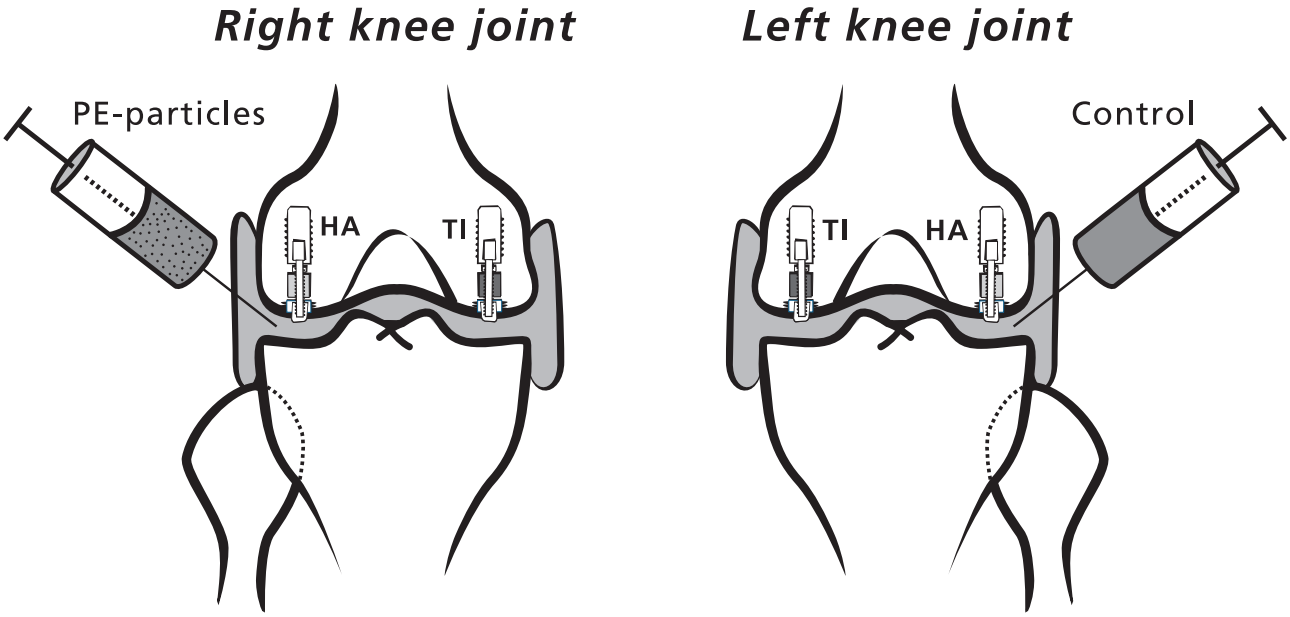

Figure 1. *Design of study I, II and III.* A weight-loaded HA-coated (HA) and a non-HA-coated grit blasted implant (Ti) were randomly allocated to medial or lateral condyle with the contra-lateral knee as control. In study III, porous-coated implants with and without HA coating were used. Three weeks after surgery, high-density polyethylene particles were injected weekly into the right knee joint. The left knee received only the vehicle (hyaluronic acid). In study II, injections were performed every second week after 8 weeks. Observation time in study I, II and III were 8, 52 and 16 weeks respectively.

10^8 particles per mL joint space. The load was doubled to accelerate the biological response and to test the shielding effect of the implants during a high particle load. The injections were started 3 weeks after surgery. The injections were performed weekly to mimic the continuous production of particles in a human artificial hip joint. In study II injections were performed every second week after 8 weeks.

Observation time was 8 weeks in study I. Based on Howie´s study [71], we expected particle mediated bone resorption to occur at that time point. Since we found no signs of osteolysis and huge difference in migration of particles, we prolonged the observation period in study II to examine if the difference in migration lasted and to see if osteolysis would occur. Thus, 52 weeks were chosen. The observation time in study III was 16 weeks to allow the implants to have bone-ingrowth and a relative long-term exposure of PE particles.

Experimental model

The implant system used has been slightly modified from an implant-device developed by Søballe et al [141;142;145;146] (Figure 2). The distal femoral epiphysis in the dog serves as implantation site. A PE plug protrudes slightly above the cartilage; hereby a load is transferred through the implant system at each gait cycle. Initially, the load is only transferred through the anchorage screw leaving the test implant unloaded, because the haematoma in the gap is unable to transmit a significant amount of shear load. However, once the gap is bridged by bone or fibrous tissue with enough tenacity to induce a load transfer to the surrounding bone, a shear load is transferred between the implant and bone. Load has been shown to increase the mechanical fixation and bony anchorage of HA implants in the Søballe implant device. However, loading did not improve the fixation of Ti implants; in contrast, the ingrowth of fibrous tissue was increased by loading [100].

We inserted the implant in trabecular bone, because most cementless total joint replacements

rely on trabecular bone ingrowth (proximal tibia, distal and proximal femur etc.). Thus, the interface initially exposed to wear debris is between trabecular bone and the implant. The gap model was chosen, since cementless prostheses intended to be inserted in a press fit will have several large areas with gaps between the implant and the bone [118;128]. These gaps are an extension of "the effective joint space" [130] and therefore potential pathways for migration of wear debris and cytokines produced in the synovial tissues.

Sample size

The error of the first kind (2α) was selected to 5% and the error of the second kind (β) was selected to 20%. We assumed the SD to be 50 % of the mean. The minimal clinical difference not to be overlooked between test groups was set to be 70 %. Based on these assumptions, it was calculated that at least seven dogs should be included in each study. Thus, eight dogs were included in study I and III and seven dogs were included in study II.

Implants

Implants were cylindrical (height: 10 mm, diameter: 6 mm), titanium alloy (Ti-6-Al-4V) implants with a grit-blasted or porous surface. The HA coating was plasma sprayed (thickness 50 μm, crystallinity 68 percent and purity 99 percent). Test implants were manufactured by Biomet Inc., Warsaw, Indiana, USA. Surfaces and coatings were manufactured as on commercial available implants. The implants were sterilized by gamma irradiation.

Study I and II: Grit-blasted implants with and without HA-coating were compared. The mean roughness (Ra) of the grit-blasted titanium surface was 1.12 (SD 0.04) μm and 1.25 (SD 0.05) μm for the HA-coated.

Study III. Porous-coated implants with or without HA-coating were compared. The porous coating was plasma-sprayed with an average porosity of 22 (SD 9) %. The mean roughness (Ra) of the porous titanium surface was 26 (SD 3) μm and 17 (SD 3) μm for the HA-coated.

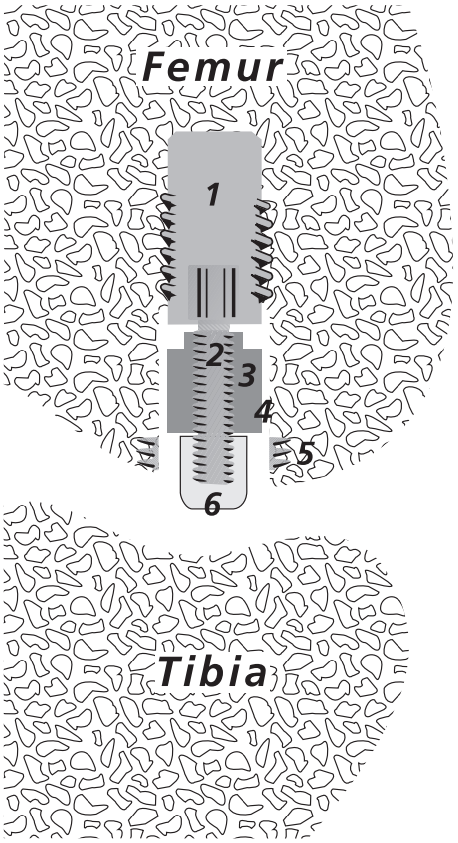

Figure 2. *Implant device and test implant positioned in the weight-bearing part of the femoral condyle.* **1.** Threaded anchorage screw fixed in bone. **2.** Threaded piston, which is centralized in the drilled hole by the anchorage screw. **3.** Test implant mounted on the piston. **4.** Gap measuring 0.75 mm between implant surface and trabecular bone. **5.** Titanium ring inserted in the subchondral part of the condyle to prevent early tissue ingrowth to the polyethylene plug. **6.** Protrusion of the polyethylene plug (diameter 4.5 mm), which transmits the load from the tibial part of the knee to the implant system. Note the access of joint fluid to the peri-implant gap. Modified after Søballe et al [141;146].

Analyses of implant roughness were conducted at the Danish Technology Institue. Four to five implants were analysed in each group with 4 measurements on each implant. The manufacturer provided the other implant characteristics.

HDPE particles

The polyethylene powder consisted of 100 percent pure crystalline HDPE (information from manufacturer). SEM (Cambridge S360) determined the particle size distribution with automatic image analysing equipment. The analysis was performed at the Danish Technological Institute. The mean equivalent circle diameter was 2.09 μm by frequency distribution and 6.3 μm by volume-weighed distribution (range 0.2-11 μm). The shape was spherical. The powder consisted of 7 percent particles with a diameter below 1 μm by frequency distribution. The HDPE resin was chosen because it contains submicron particles as found around loose implants [136] and an inflammatory response has been shown experimentally after only six weeks of stimulation with HDPE particles with approximately the same mean size (2.03 μm) [5]. Particles from hip simulators were also considered to be used, however, such particles would contain only few particles larger than 1 μm and sub-micron particles below 0.7 μm in diameter are not detected by light microscopy, unless they are multiple and producing "Willert´s Phenomenon" [165].

The particles were gamma-sterilised. The particles and hyaluronic acid were analyzed by gas chromatography-tandem mass spectrometry. This analysis is highly sensitive for trace levels of lipopolysaccharide (endotoxin) [140]. There were no biochemical markers for endotoxins in any of the samples tested.

Hyaluronic acid

Hyaluronic acid was selected for carrier of the particles for a number of reasons. First of all, hyaloronic acid is a natural component of joint fluid contributing to the high viscosity of the fluid. It is commercially available as powder in a pure form produced by strains of Streptococcus zooepidemicus. The advantage of the powder is that the particles and the powder can be mixed, before the phosphate-buffered saline water is added. The high viscosity of hyaluronic acid keeps the particles dispersed in the solution. Pilot-studies showed that a concentration of 1.75 mg hyaluronic acid/ml phosphate-buffered saline provided us with a viscosity, which allowed us to keep the particles dispersed in the solution and at the same time allowed us to suck the solution into a syringe afterwards.

Studies have shown that hyaluronic acid may accelerate bone healing [127] and inhibit the degenerative changes in cartilage osteoarthrosis and chondromalaci [137;161;171]. However, the effect of peri-implant hyaloronic acid has been tested in the Søballe implant micromotion device and in an extra-articular gap model [15;121]. No effect was demonstrated by hyaloronic acid on morphology and mechanical properties of the interfacial tissue compared with an empty gap.

Postoperative registration

All dogs were allowed full weight bearing postoperatively. All dogs walked and supported all four limbs at the third day postoperatively. The dogs were inspected regularly. Animal care was done under identical conditions in individual cages. Daily outdoor activities were allowed.

Post-mortem, the knees were opened under sterile conditions and cultures were taken from the joints in study I. Some bacterial cultures showed scattered growth of Staphylococcus albus, probably because of contamination during sampling, since no clinical signs of infection were noticed. In study II and III cultures were not taken because of the conflicting results in study I. In all cases no signs of infection were present neither clinically nor histological in synovial biopsies.

Preparation of sections

Each bone-implant specimen was split in halves and one half was furthermore parted into quarters according to figure 3. Sections from the undecalcified block were approximately 25-30 μm thick and were counterstained with 4 percent light green [63]. Sectioning was performed on a microtome (KDG-95, MeProTech, Netherlands) with 350 μm between sections.

By random one part containing one quarter

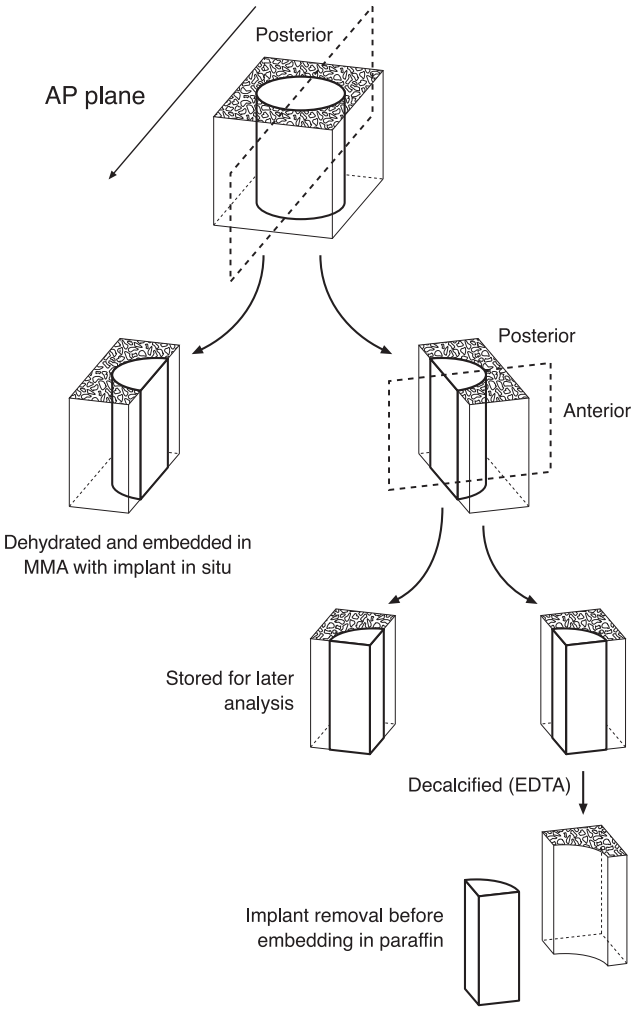

Figure 3. *Preparation procedure.* Cutting the implant vertically through the middle produced a medial and lateral block. By random one half remained undecalcified and was dehydrated in graded ethanol (70-100 percent) containing basic fuchsin and embedded in methylmethacrylate. The other half was cut vertically through the middle of the implant. Hereby a posterior and anterior block were produced each containing a quarter of the implant. By random one part was stored in formaldehyde and decalcified (EDTA). The implant was gently removed from the surrounding tissue, which hereafter was paraffin embedded. The remaining part was stored in 70 percent alcohol for a possible later analysis and is not included in this work.

of the implant was stored in formaldehyde, decalcified (EDTA) and embedded in paraffin. Paraffin embedded material was cut in 7 μm thick sections with 200 μm (in study I: 50 μm) between sections. Two sections were cut for each step and stained with HE and Oil Red O [67;131] respectively.

Histological and histomorphometric analysis

Histological evaluation was done qualitatively by simple description, semi-quantitatively by rating of particle migration and quantitatively by histomorphometry.

An Olympus BX 50 microscope modified for stereology was used. A video camera was attached to the microscope and hereby the microscopic field is transmitted to a computer monitor. A stereological software program was applied (CAST-Grid®, Olympus Denmark A/S) for histomorphometry and rating of particle migration. This is based on a user-specified grid or counting frame super-imposed on the microscopic field captured on the monitor. We used objective x 10 and ocular x 10 for all analyses. The depth of focus with plain unfiltered light is approximately between 3.0 and 3.8 μm. The resolution of conventional light microscopy is 0.4-0.7 μm and is determined by the wavelength of visible light.

Cutting of paraffin embedded tissue required the removal of the implant after decalcification of the bone-implant specimen. This removal did not cause any problems with grit-blasted or HA-coated implant surfaces (Study I & II). The hydroxyapatite coating was easily detached from the implant at the metal-HA interface after decalcification. However, in study III we had difficulties with porous-structured surface coatings. The peri-implant tissue had grown into the pores and was firmly attached to the coating. Therefore, the peri-implant tissue was in a few cases destroyed by the removal of the implant. MMA embedded sections were therefore used for the rating of PE particles in study III.

Table 3. The table shows how sections were rated in respect of inflammation and marrow necrosis. These ratings were used in study II and III. Inflammation and marrow necrosis were not seen in any sections in study I.

Rating of sections	Inflammation	Marrow necrosis
0	No mononuclear cells present	No areas of marrow necrosis
1	One small infiltrate of mononuclear cells	One necrotic area
2	More than 1 infiltrate of mononuclear cells	More than 1 area of necrosis
3	Chronic inflammation dominating the interface	Marrow space dominated by necrosis

Inflammation and marrow necrosis

Inflammation and marrow necrosis in the peri-implant tissue were rated in study II and III (Table 3). The rating was done blinded by a pathologist on HE stained sections.

Identification of polyethylene particles

Particles were identified at a magnification of x 100. At this magnification the majority of the injected particles were easily identified. However, some information was lost about the sub-micron particles, mainly because of the resolution of light microscopy. PE particles in the histological sections were defined as follows:

Decalcified paraffin embedded sections (Study I and II). Only ORO stained sections were used. Sections were examined by use of polarized light (lambda filter) at the maximum light setting on the microscope. A red stained (ORO-stain) and birefringent dot or flake was identified as a PE particle.

MMA embedded sections (Study III). Only the first focus plane was analyzed. A birefringent dot or flake at the maximum light setting on the microscope was defined as a PE particle. However, some birefringent particles were not counted. We found the glue could contain some birefringent particles, however these particles were not in the same focus level as the tissue. Inside the tissue glove particles could be found to polarize, however, these particles were easily recognizable.

Sensitivity and specificity. To ensure that PE particles could qualitatively be detected on paraffin and MMA embedded sections, the area around the PE plug was analyzed blinded on sections from 32 different bone-implant specimens. The area around the PE plug was selected for this analysis, because the PE plug processed little sealing effect and was surrounded by a fibrous membrane. If particles were injected into the knee joint they would also be present in this area. The area was rated yes or no regarding the presence of polyethylene particles. Furthermore this analysis tested our model. To allow us to test implant surfaces in relation to PE particles, it is necessary that particles have access to bone-implant interface (Figure 9.c. and 10.c.). Thus, particles had to be present in the area around the PE plug, if injected into the joint space. In this way, this blinded analysis also served to validate our model.

In all ORO stained sections from PE-injected knees polyethylene particles were found. No particles were found in sections from control knees. Small artifacts of ORO stain could be seen in some sections. These were probably due to dye, which had not been removed during the staining procedure. Furthermore, intracellular staining could be seen in some sections. However, these artifacts were easily distinguished from PE particles because they were not birefringent.

When MMA embedded specimens were analyzed, PE particles were also easily identified in all sections from PE-exposed knees and no particles were found in control knees.

Thus, by analyzing sections with polarized light we found no difference in sensitivity and specificity between ORO-stained paraffin embedded sections and MMA sections.

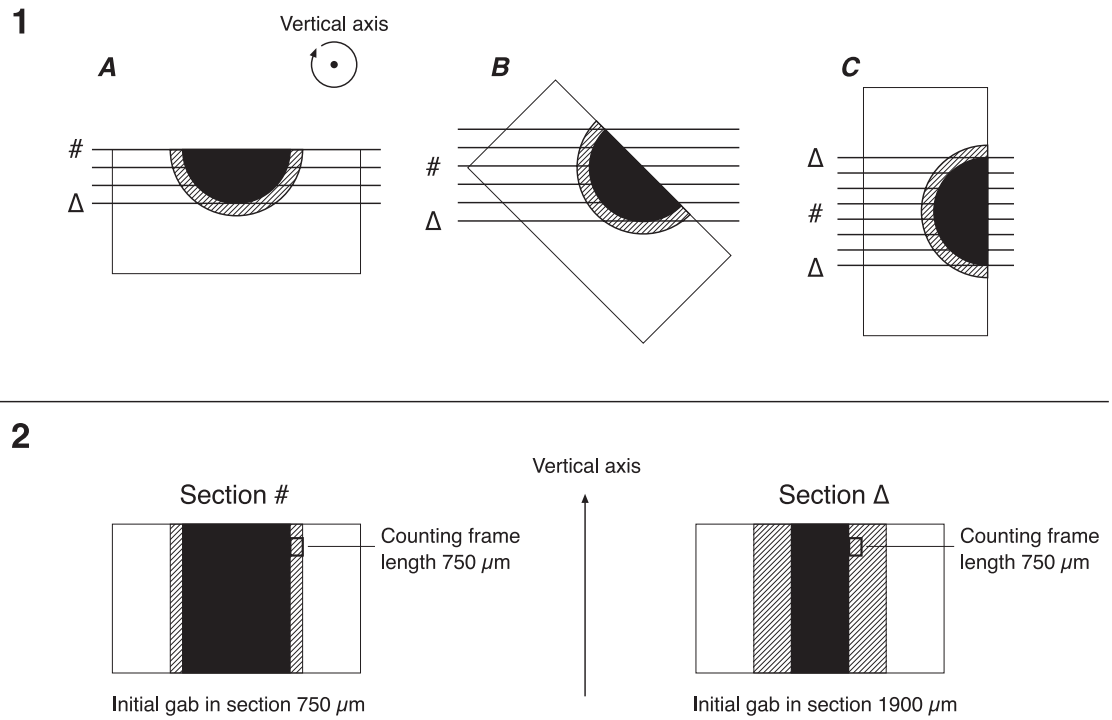

Figure 4. *Effect of section level.* **1.** This figure illustrates the sectioning of the MMA embedded bone-implant specimen. A, B and C illustrate three examples of, how the bone-implant specimen is cut after random rotation around the vertical axis. The section level determines how the initial gap is represented in the section. The rim of the initial gap will lie in a distance ranging from 750 µm (sections marked #) to 1900 µm (sections marked Δ) from the implant surface. The figure also illustrates that the gap in most sections is close to 750 µm. In the present studies, we estimated gap healing and particle migration from sections, which cut the implant perpendicular, tangential or in between. Since the rotation and section level were random, the estimate was not biased. **2.** The initial 750 µm gap was only represented in its original dimension in sections marked #, where the section was cut in perpendicular to the implant surface. In section marked Δ, only one third of the initial gap was analysed in the tangential plane of the implant surface.

Furthermore, we found that the injected particles had access to the bone-implant interface.

Principles for efficient and unbiased estimates

In order to obtain efficient and unbiased estimates of histomorphometric parameters, stereological principles were followed, and systematic sampling was applied. By efficiency is understood that the estimate is obtained with a low variability after spending a moderate amount of time, and unbiasedness means without systematic deviation from the true value [66;115].

By using stereological methods, information about three-dimensional structures can be obtained from two-dimensional sections. Trabecular bone is an anisotropic structure, which means that it has a preferred orientation [162]. When volumes and numbers are estimated from two-dimensional sections this raises no problems, but when surfaces are estimated, bias is introduced by the anisotropic orientation of trabecular bone. The vertical sectioning method [12] was introduced for bone-implant research by Overgaard et al [114] to solve this problem. Four requirements must be met to obtain unbiased estimates of surface. 1) The vertical axis has to be defined. In the case of cylindrical implants, it is easy and seems natural to choose the long axis of the implant. 2) The specimen has to be rotated randomly around the vertical axis before sectioning. 3) Serial cut random positioned sections must be performed parallel with the vertical axis. 4) Finally, the vertical axis must be visible in every section. A set of isotropic uniform random IUR test lines is then applied in the microscopic field of vision. The IUR test lines are given by a weight proportional to the sine of the angle between the test line and the vertical axis. Together with the

initial random rotation around the vertical axis, the lines are now distributed uniformly and randomly in three-dimensional space.

To improve efficiency we applied systematic sampling. The KDG-95 hard tissue microtome allowed us to cut serial sections with a thickness of 25-30 μm spaced by 350 μm between sections. The efficiency of systematic sampling has been studied by Overgaard et al on bone-implant specimens from humans [115]. It was concluded that the contribution of the section level to the total observed variance was low as compared wtih the true biological variance. The workload could be reduced by analysing only 3-4 uniform random sections per implant without affecting the total variance greatly. However, the biological variance may be lower in the present studies, because the inbred dogs represent a more genetically homogeneous group of individuals than the group unrelated humans. Still the biological variation must be considered relatively large. Hence, mean values of 5, 5.6 and 4 sections were analysed in study I, II and III respectively.

There are some limitations within the use of vertical sectioning of bone-implant specimen halves with regard to gap healing. When the bone-implant specimen is cut serial, the rim of the initial 750 μm gap will lie in a distance ranging from 750-1900 μm from the implant surface depending on the section level (Figure 4).

Migration of particles

Migration of PE particles was evaluated by use of a counting frame (750 x 1100 μm). The counting frame was aligned to the implant surface so the peri-implant tissue in the range of 750 μm from the surface was analysed. The peri-implant tissue around the implant was divided into eight zones with a height of 1100 μm (Figure 5). The number of PE particles inside the counting frame was rated according to a grading system (Table 4).

Bone ongrowth and gap healing

Linear intercept technique and point counting was applied to estimate bone ongrowth and gap healing. By using linear intercept technique sine-weighted straight test lines were superimposed on the microscopic field. When the IUR lines cut the implant surface the type of tissue in contact with the implant was registered. By point counting the type of tissue hit by point was registered.

As a rule-of-thumb, 100-200 events of interest have to be counted; hereby the contribution to the total variance from the fields of view is 7-10 % ($CE = 1/\sqrt{n}$) given a sufficient number of fields of vision are sampled [66].

Figure 5. This simplified diagram shows a histological section with an implant. The counting frame is moved stepwise from the juxtaarticular tip of the implant to the base of the implant, thus dividing the periimplant area into eight zones.

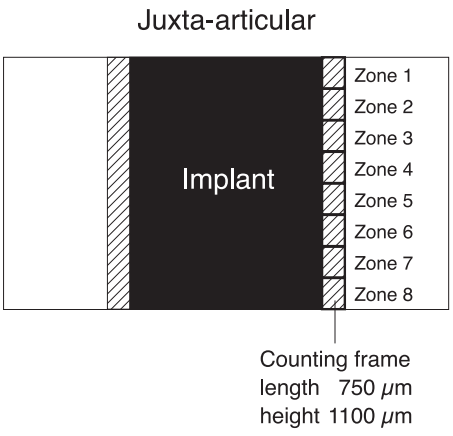

Table 4. Grading system for PE particle migration modified after Mirra [99] et al and Hicks et al [69]

Number of particles per counting frame	Grade
0 particles	0
1-9 particles	1
10-19 particles	2
20-49 particles	3
≥ 50 particles	4

Reproducibility

Intra-observer agreement on the ratings of number of PE particles

105 fields were graded twice for both decalcified paraffin sections and MMA sections respectively. The ratings were performed with approximately 2 days apart.

Decalcified paraffin embedded samples (Table 5). No systematic error between ratings could be found. The average disagreement was -0.15 (range -1--2). Percentage of acceptable disagreements was 28 % and the percentage of severe disagreement was 1 %. Disagreements were mostly found for grades 1-3. Kappa value was 0.66.

MMA embedded samples (Table 6). The average disagreement was -0.15 (range -1-1). Like for the decalcified sections disagreement was mainly found for grade 1-3. Percentage of acceptable disagreements was 26 %. No cases of severe disagreement were found. Kappa value was 0.65. A systematic error between ratings was not found.

Generally we found that grade 1 and 2 were the ratings, which had the highest level of disagreement, when fields were rated twice. This might be because the interval of number of particles being very narrow for these ratings. So if 1 or 2 particles are included or excluded, maybe due to a slightly different placement of the counting frame, this may very well change the rating one grade up or down.

The type of section used did not seem to influence the precision of the rating. We found similar Kappa values for intra-observer agreement on approximately 0.65 for both types of sections. This is commonly referred to as good agreement [6], but it should be kept in mind that acceptable agreement depends on circumstances.

Kappa values depend on the number of categories used. We have five categories in the presented grading system. If data were divided into two grades, for example <20 and ≥20 particles per counting frame, Kappa value could be increased to 0.79. However, by gaining a higher agreement, information may be lost and relevant differences in sealing effect may not be detected.

Table 5 & 6.
Frequency table on double-rated fields for decalcified paraffin embedded sections and MMA embedded sections respectively.
Acceptable disagreements were defined as ratings that differed one grade from the row or column value. Severe disagreement was defined as number of ratings, which differed more than 1 grade from the row or column (gray area).

Table 5. Paraffin sections		1. rating					
		0	1	2	3	4	Total
2. rating	0	16	8				24
	1	1	16	3			20
	2		4	12	2		18
	3		1	5	18	1	25
	4				4	14	18
	Total	17	29	20	24	15	105

Table 6. MMA sections		1. rating					
		0	1	2	3	4	Total
2. rating	0	20	4				24
	1	9	17	2			28
	2		7	7			14
	3			3	8		11
	4				3	25	28
	Total	29	28	12	11	25	105

Agreement of ratings between paraffin sections and MMA sections

In order to analyse the agreement of rating between the paraffin embedded and MMA embedded block from the same bone-implant specimen, the median values of all fields rated per specimen were compared for 21 bone-implant specimens (Figure 6).

The direct comparison between MMA embedded sections and paraffin embedded sections from different blocks of the same bone-implant specimen can, however, be questioned. The best comparison would be acquired by cutting the block alternating between paraffin embedded and MMA embedded sections. However, this was not technical possible. We had to assume the particles to be equally distributed around the implant, since the sections were not cut from the same block of the bone-implant specimen.

The thickness of the sections might have influenced the rating of particles, because MMA specimens were rated significantly higher than paraffin embedded sections. Since we were operating with a depth of focus between 3 and 4 μm, the thickness of the sections theoretically should not matter, because they were all thicker than 4 μm. However, when polarized light is used, it can be hard to tell if a particle is in focus or not, especially in the presence of multiple particles. Therefore, particles lying close to the focus plane may also be counted. Nonetheless, one would expect the scores of the bone-implant specimens to correlate and indeed the results showed a good correlation between data from MMA and paraffin sections.

Intraobserver agreement on bone ingrowth and gap healing

Double measurement on 12 implants revealed a coefficient of variance on 9 and 11 percent for bone ingrowth and bone healing in the initial gap respectively. Measurements were done with a few days apart. These intra-observer agreements were at an expected level [66] and in accordance with previously published CE on these parameters [110;141].

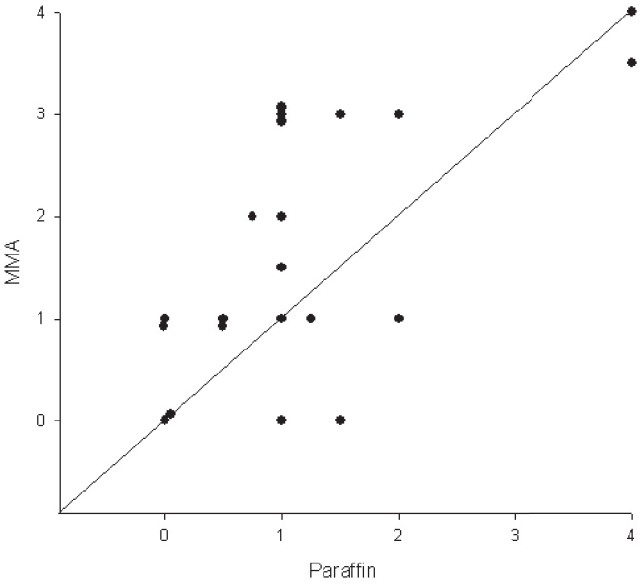

Figure 6. *The rating of particle migration on MMA sections plottet against the rating on paraffin sections.* Eight fields were rated once on a mean of 3.3 (range 2-4) consecutive sections of 21 decalcified paraffin embedded blocks. Likewise, eight fields were graded on a mean of 3.7 (range 2-4) sections on the MMA embedded counterpart of the specimen. Median values are plotted. Many specimens that were rated 1 on paraffin sections were rated quite differently on MMA sections. No specimens were graded between 2 and 4 by the use of paraffin embedded sections. When MMA embedded sections were used, the ratings were equally distributed between 0 and 4. MMA bone-implant specimens were rated significantly higher with a median value of 1.63 as compared with 1.00 for paraffin embedded sections (p=0.012). Ratings on the same bone-implant specimen with the two types of sections were correlated with ρ = 0.75 (p = 0.01).

Statistics

In study I parametric testing was applied by paired or unpaired *t*-test. In study II and III non-parametric testing was used, because data did not have a Normal distribution. Significance was thus determined by the Mann-Whitney rank sum or Wilcoxon´s signed rank test. Spearman´s ρ was used for non-parametric correlations. Two-sided p-values less than 0.05 were considered significant.

Data for all studies are presented as median values and range, allowing the reader to compare data. In figures inter-quartile ranges are presented.

Results

Missing samples

Some tissue samples were lost for histological examination during the histological preparation of the decalcified sections. In study I, interfacial tissue was lost from a PE-exposed Ti implant. In study III, one interfacial tissue was lost from the HA-po/+PE group. Furthermore, two interfacial tissues were lost for analysis from the group of Ti-po/+PE and Ti-po/-PE each.

Morphology of peri-implant tissue

PE-plug

In all studies performed, we found a fibrous membrane surrounding the PE plug. Infiltration of lymphocytes, macrophages and plasma cells was seen around the huge amounts of PE particles in PE-exposed knees.

Ti implants

A thin fibrous membrane with a lining of synovial-like cells surrounded Ti implants. The layer beneath the synovial lining was rich in extracellular matrix with fibers orientated parallel with the implant surface. In this layer scattered spindle-shaped cells predominated. The deep layer of the membrane close to the bone was rich in capillaries and larger vessels. The membrane was in most cases bordered with a line of sclerotic bone. Large areas with scalloping of the bone surface and osteoclasts were not seen.

After 8 weeks (study I), membranes from PE-exposed knees had the same morphology as membranes from control knees except from the presence of PE particles. Around Ti-po implants (study III), areas with ingrowth of bone were seen in some cases after 16 weeks (5 of 16 implants). Furthermore, small areas of inflammation were noted around a few implants. Areas of inflammation were equally distributed between the PE-exposed and control group.

After 52 weeks an inflammatory reaction was seen around 3 out of 7 implants in the PE injected group compared with none in the control group. For those interfaces without inflammation, the membrane resembled those from the control group with the exception that the membranes contained huge amounts of PE particles (Figure 7). The inflamed membrane was dominated in the worst case by a huge amount of mononuclear cells and with areas of necrosis. Lymphocytes, macrophages and plasma cells predominated (Figure 8). Formation of foreign body giant cells was not seen in any of the sections.

Examination of the bone marrow between implant and cortical bone revealed after 52 weeks necrotic areas with a myxoid matrix. No signs of inflammation were present around these areas and only a few PE particles were found around these lesions. There was no significant difference comparing PE-exposed and control groups for both HA and Ti implants separately ($p=0.26$ and $p=0.053$). If all implants were grouped in two groups (n 14), a significant difference was found between PE exposed and control implants ($p=0.025$), suggesting that the PE particles might be involved in the pathogenesis of marrow necrosis.

HA implants

In all studies, HA-coated implants were surrounded by bone marrow with growth of trabecular bone into the HA-coating. After 8 weeks, we found mainly woven bone, whereas after 52 weeks mature lamellar bone was seen. Synovial lining cells were not present along the implant surface. Fibrous tissue was a rare finding and mostly seen in areas with delaminated coating. No major inflammatory reaction was noted in any of the studies. In study III, small areas of inflammation were noted around a few PE-exposed and control implants.

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Figure 7. *Interface from PE exposed Ti implant, study III (52 weeks).* Note the benign appearance of the membrane. The membrane contains few spindle-shaped cells and scattered particles (arrows). Oil red O stain, x 100.

Figure 8. *Interface from PE exposed Ti implant, study III (52 weeks).* Note the loose tissue with infiltration of round cells. PE particles are scattered in the tissue (arrows). Oil red O stain, x 100.

Figure 9.a. *PE exposed Ti implant, study III (52 weeks).* Stained with light green and basic fuchsin. Bone is green and soft tissue is red. Note the fibrous membrane extending from the level of the PE plug (empty space left to the implant) to the base of test-implant. The two superimposed frames shows the location of figure 9.b and c. Plain light-microscopy, x 12.5.

Figure 9.b. *PE exposed Ti implant, study III (52 weeks).* Microscopic field of the fibrous membrane around PE plug. The juxtaarticular tip of the Ti implant is seen to the right. Numerous of PE particles are distributed in the membrane and marrow space (arrows). Basic fuchsin and light green. Polarised light microscopy, x 40.

Migration of PE particles in the bone-implant interface

A marked effect of HA on particle migration was found around grit blasted implants after 8 weeks. The effect lasted even after 52 weeks (Figures 9.a.b.c and 10.a.b.c). The pattern of migration in the interface differed between Ti and HA-coated implants. Implants from the HA group had significantly fewer particles compared with Ti implants (Figure 11). In the group of Ti implants, the amount of particles was equal in all zones. In contrast, there was significantly more particles in the juxta-articular zone compared with the most proximal zone for HA-coated implants ($p = 0.001$).

A less marked, but still significant effect, had HA applied on porous coated implants (Table 6). By looking on the distribution of particles in the eight peri-implant zones we found only significantly fewer particles around HA-po implants in the mid-implant zones 4 and 5 compared with Ti-po implants (Figure 12). If data were grouped in 4 zones instead of 8 zones, HA implants had significantly fewer particles in the 3 most juxta-articular zones. For the last zone at the basis of the implant, a p-value of 0.058 was found. Presence of PE particles around Ti-po implants was graded high in the zones near the joint space. Median ratings of particles decreased in the zones along the Ti-po implants towards the base of the implant ($\rho = -0.95$, $p < 0.01$). HA-po implants had a median value of approximately 1 in all zones (Figure 12).

Table 6. *Results from study I, II and III on migration of PE particles.* **a:** evaluated qualitatively **b:** rated on thin ORO stained sections **c:** rated on thick MMA embedded sections.

Study	HA implant	Ti implant	p-value
I [a] (grit-blasted, 8 weeks)	Few scattered particles	Large amounts of particles in the entire interface	-
II [b] (grit-blasted, 52 weeks)	1 (0-1.5)	4 (1.5-4)	0.001
III [c] (porous-coated,16 weeks)	1 (0-1)	2 (1-4)	0.01

Figure 7

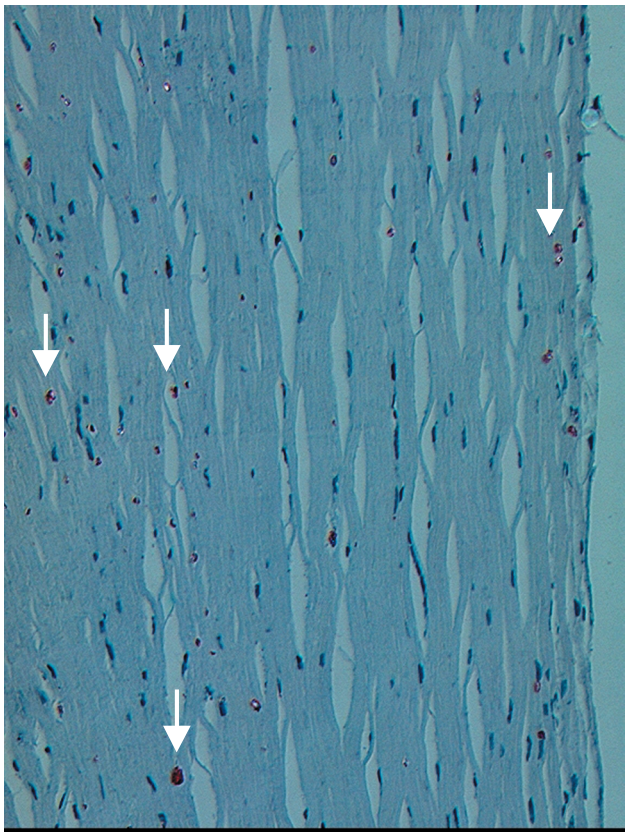

Figure 8

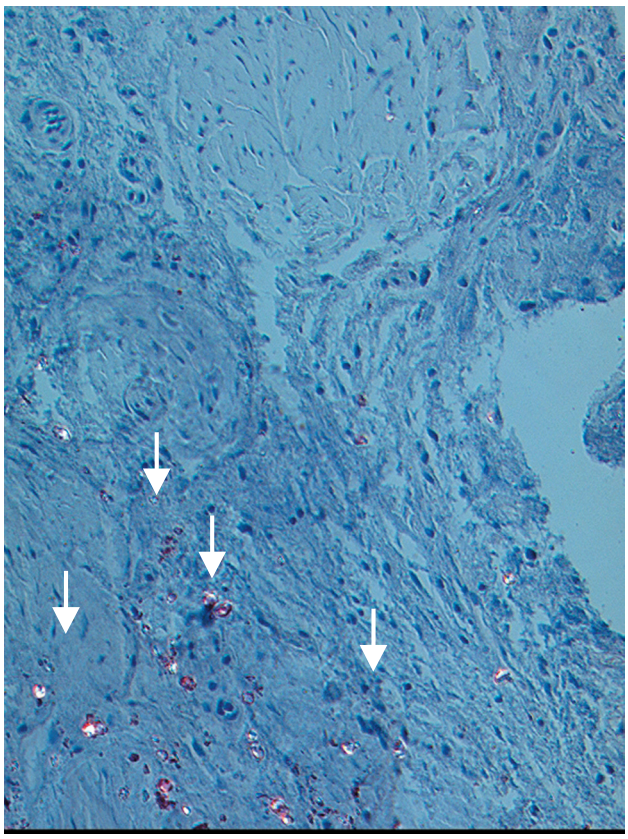

Figure 9.a

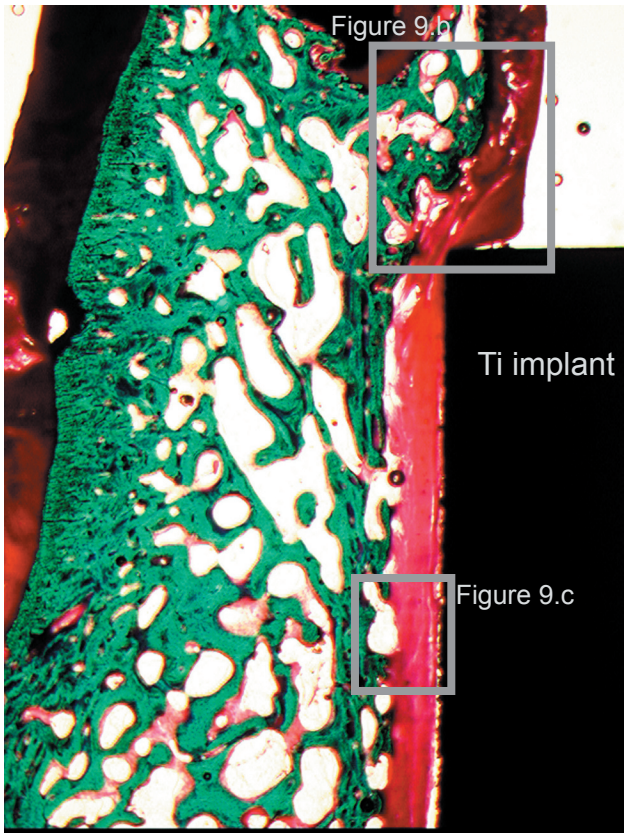

Figure 9.c

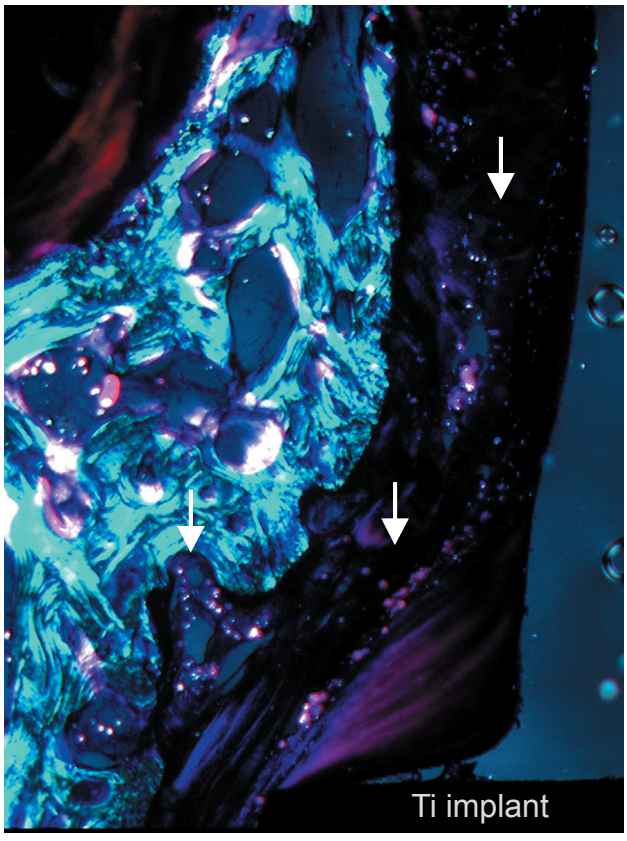

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Figure 9.c. *PE exposed Ti implant, study III (52 weeks).* Microscopic field of the fibrous membrane at mid-implant level of Ti implant. Multiple PE particles scattered in the membrane (arrows). Basic fuchsin and light green. Polarised light microscopy, x 100.

Figure 10.a. *PE exposed HA implant, study III (52 weeks).* Bone-implant interface sealed off by bone. The two superimposed frames show the location of figure 10.b and c. Plain light-microscopy, x 12.5.

Figure 10.b. *PE exposed HA implant, study III (52 weeks).* Multiple PE particles are present in the fibrous membrane around the PE plug having access to the implant interface. Note sealing of the entrance to the bone-implant interface. Basic fuchsin and light green. Polarised light microscopy, x 40.

Figure 10.c. *PE exposed HA implant, study III (52 weeks).* HA implant at the mid-implant level. Note the intimate contact between bone and HA. Few PE particles are seen (arrows), which demonstrates the sealing effect by HA. Basic fuchsin and light green. Polarised light microscopy, x 100.

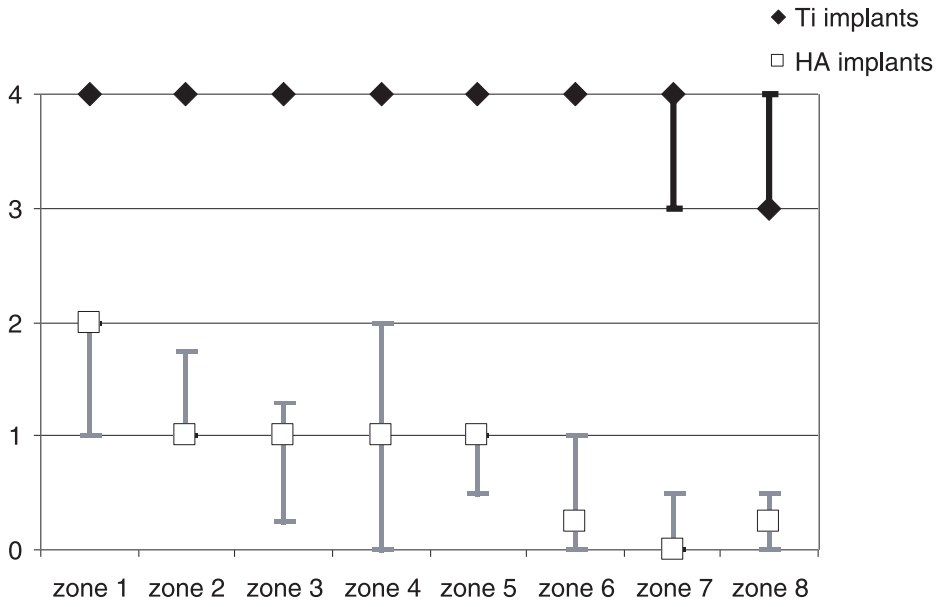


Figure 11. *Migration of PE particles after 52 weeks around grit-blasted implants.* Median values of particle rating with quartiles are displayed for each zone. PE particles were rated significantly lower in all zones.

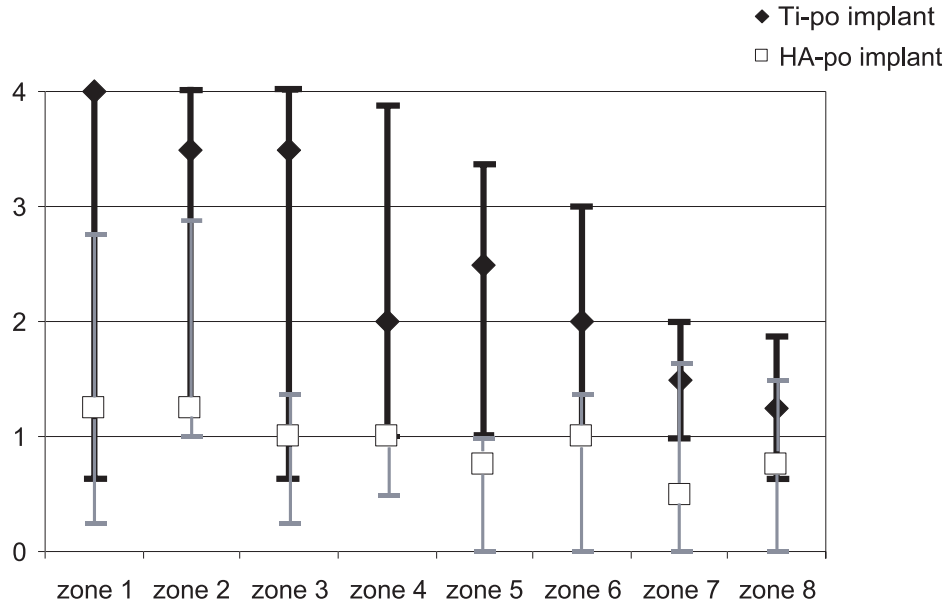


Figure 12. *Migration of PE particles after 16 weeks around porous-coated implants.* Median values of particle rating with quartiles are displayed for each zone. P-value < 0.05 was found for zone 4 and 5.

Figure 9.c

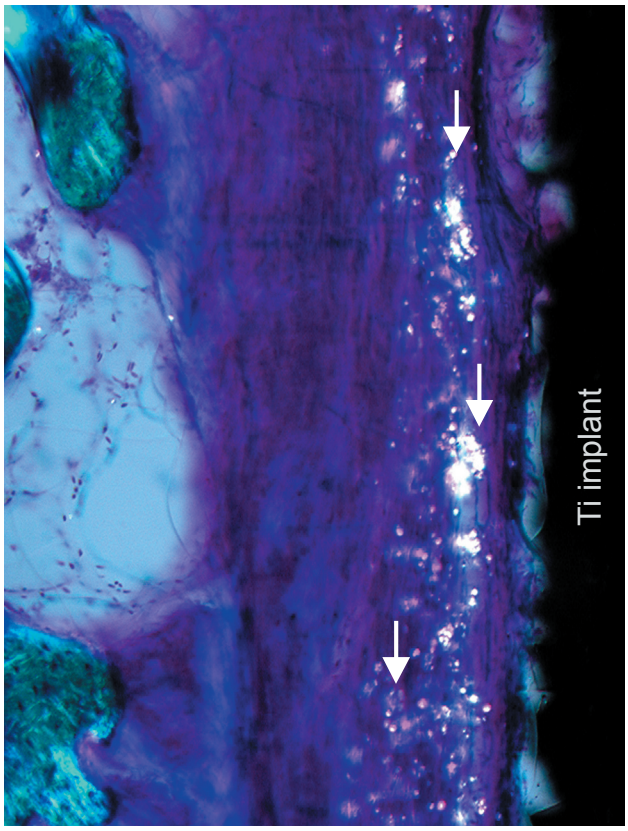


Figure 10.b

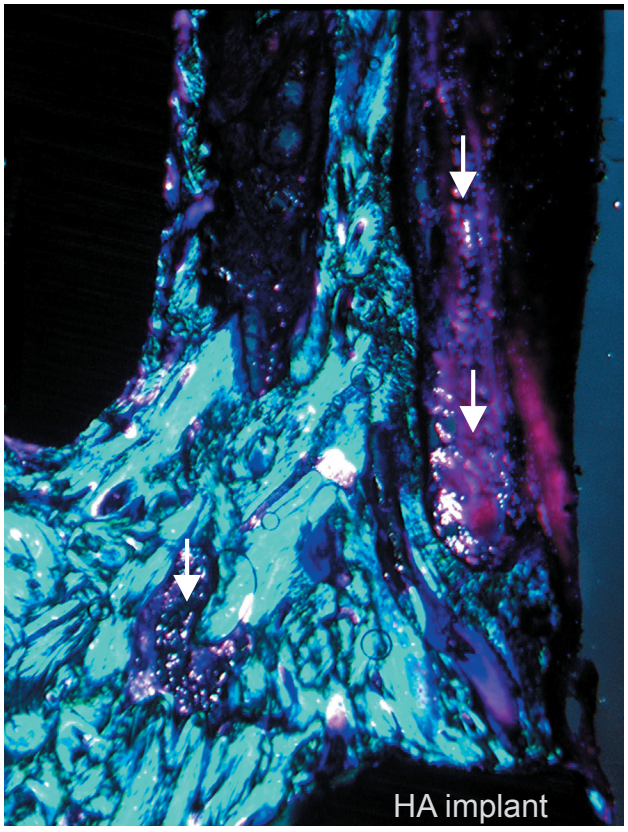


Figure 10.a

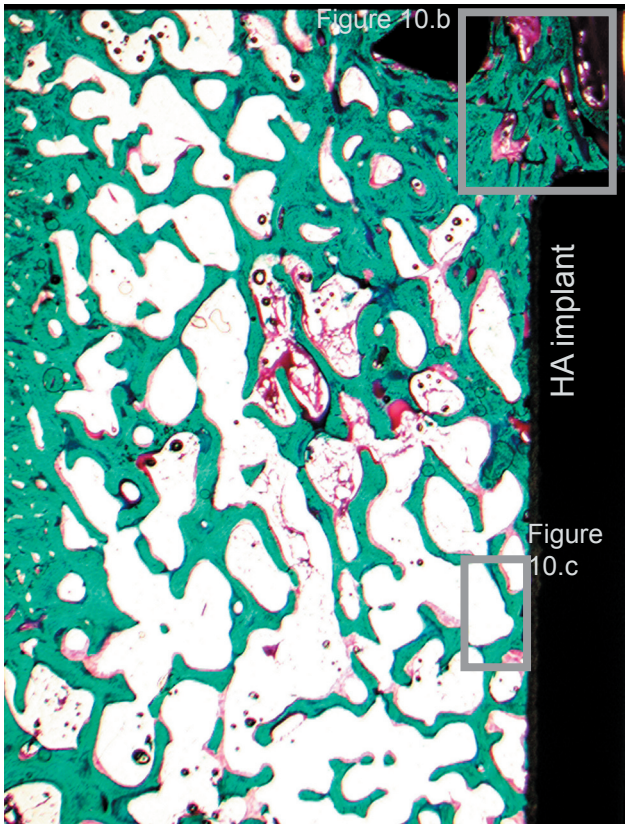
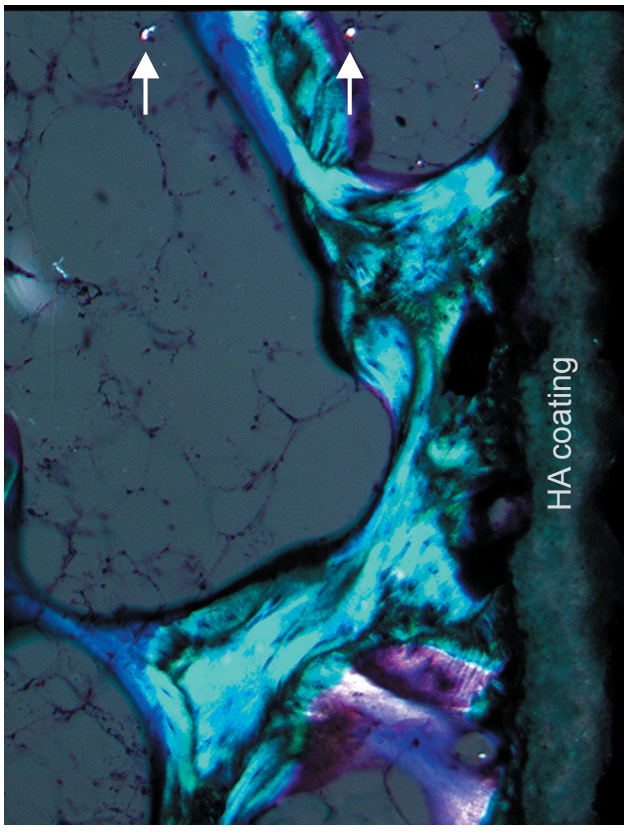


Figure 10.c



Bone ongrowth and gap healing

PE particles did not influence the healing around implants. Ti implants were covered with a fibrous membrane even after 52 weeks. The volume of the fibrous membrane in the initial gap was also unchanged by the presence of particles. In some cases Ti-po implants had bone coverage up till 30 %.

Implants with HA coating had significantly more bone ingrowth. The effect of HA increased by time and bone coverage of 70-75% was present after 52 weeks. Thirty-six percent of the bone-ingrowth was gained within the first 8 weeks (Table 7 and 8).

Synovial biopsies and distant organs

Histological examination showed no signs of infection. In the particle-exposed knees, PE particles were located beneath the synovial lining cells along with lymphocytes and plasma cells. Inflammation was only present in the superficial part of the synovium. Multinucleated foreign body giant cells surrounded some of the particles.

Examination of the right-sided medial iliac lymph nodes with polarised light and ORO staining revealed a large number of PE particles distributed in both the cortical and medulla areas, but none on the left side. Biopsies from spleen, lungs and liver showed no PE particles even after 52 weeks.

Table 7 & 8. *Histomorphometric results from study I, II and III.* **a:** No significant difference compared with control **b:** Significant different compared with Ti implant ($p<0.002$) **c:** No significant difference compared with Ti implant

Table 7. Ongrowth / Ingrowth (%)		Right knee joint/ PE particles		Left knee joint/ Control solution	
		HA implant	Ti implant	HA implant	Ti implant
Study I (grit-blasted, 8 weeks)	**Bone**	36 (27-47) [ab]	0 (0-2) [a]	36 (27-47) [b]	0 (0-5)
	Fibrous tissue	1 (0-16) [ab]	100 (30 –100) [a]	8 (0-27) [b]	98 (52-100)
Study II (grit-blasted, 52 weeks)	**Bone**	75 (27-79) [ab]	0 (0-5) [a]	71 (49-73) [b]	0 (0-1)
	Fibrous tissue	0 (0-34) [ab]	100 (95-100) [a]	0 (0-6) [b]	100 (99-100)
Study III (porous-coated 16 weeks)	**Bone**	62 (44-74) [ab]	1 (0-32) [a]	64 (46-74) [b]	6 (0-31)
	Fibrous tissue	0 (0-13) [ab]	87 (41-100) [a]	1 (0-21) [b]	76 (25-100)

Table 8. Gap healing (%)		Right knee joint/ PE particles		Left knee joint/ Control solution	
		HA implant	Ti implant	HA implant	Ti implant
Study I (grit-blasted, 8 weeks)	**Bone**	27 (16-38) [ab]	14 (2-24) [a]	23 (13-36) [b]	12 (7-19)
	Fibrous tissue	3 (0-14) [ab]	62 (13-98) [a]	8 (0-15) [b]	75 (33-86)
Study II (grit-blasted, 52 weeks)	**Bone**	36 (27-42) [ab]	7 (0-40) [a]	38 (29-56) [b]	16 (5-31)
	Fibrous tissue	0 (0-35) [ab]	86 (18-100) [a]	0 (0-12) [b]	81 (62-94)
Study III (porous-coated 16 weeks)	**Bone**	37 (19-44) [ac]	17 (11-52) [a]	38 (29-56) [c]	16 (5-31)
	Fibrous tissue	1 (0-16) [ab]	55 (21-83) [a]	0 (0-12) [b]	81 (62-94)

Discussion

Biological effects of PE particles in peri-implant bone

A striking finding in the presented studies was the lack of reaction due to PE particles in the bone implant interface. Especially, because an earlier study on rats found inflammation and bone resorption caused by similar sized particles [5]. Furthermore, we found in synovial tissue and in the area around PE plug a marked cellular response due to the particles.

Nonetheless, the fibrous membranes surrounding Ti implant had a similar morphology, regardless PE particles being present in the tissue or not. After 52 weeks inflammation was noted around some implants exposed to PE particles, however, this finding was not significant.

Our data support that bone tissue is slow in generating an inflammatory response compared to synovial tissue [106] and these findings are in accordance with earlier studies conducted by Aspenberg et al [10;11;156;157]. They found that HDPE particles did not cause bone resorption in a stable interface and demonstrated that micromotion or fluid pressure induced bone destruction more potently.

However, the data in the literature are conflicting. The first experimental study on the influence of PE particles around implants was published by Howie [71]. His model created a bone-cement interface by inserting a non-loaded acrylic plug into the distal femur of a rat. Bone resorption in the bone-cement interface was induced after only 8 weeks of repeated intraarticular injections of powdered HDPE. Since the study by Howie, several *in vivo* studies have demonstrated similar findings of particle-induced bone resorption by using small controlled experimental implants [5;22;82]. However, other studies, including our own, have failed to reproduce the findings of osteolysis [15;52;89;155].

A simplified and successful approach for inducing osteolysis, has been to surgically implant particles on the calvaria of a rat [172]. Hereby an intense inflammatory reaction and bone resorption are reported to occur within 10 days.

More clinical models, but less controlled, exist with THA´s in dogs. In these studies, radiological or histological signs of aseptic loosening have been provoked by adding wear debris-like material into a peri-implant gap [46;133].

Until recently, these conflicting results could not be explained. How could particles with approximately the same size, morphology, and number elicit so different responses ranging from a benign to rapid intense inflammation with osteolysis in experimental studies?

The answer may very well lie in an uncontrolled variable; the contamination of particles by endotoxins. As mentioned in the introduction, endotoxins have the same effects on bone as ascribed to wear debris.

Ragab et al developed a protocol by which the endotoxins can be removed from the particle surface [119]. By doing this, it was demonstrated that the cytokine production, osteolysis and osteoclast differentiation induced by particles were significantly reduced or omitted [3;9;19;41]. Considering these results, most experimental research until today could be biased, since the level of endotoxin contamination of the particles was not reported. Furthermore, if the analysis was reported, it had mostly been performed with an assay on the supernatant from a particle suspension. This approach detects only one percent of the endotoxins adherent to the particle surface [119].

Thus, the rapid aggressive response on particulate material seen in many experimental studies could be explained by endotoxin contamination. Furthermore, the more benign response in the present studies could be explained by these findings, since both carrier and particles were tested negative for endotoxins by gas chromatography / tandem mass spectrometry.

However, wear debris may still play a major role

in aseptic loosening of orthopaedic implants. It seems unlikely that endotoxin contamination plays a major role in late aseptic loosening, considering the rapid and aggressive response caused by endotoxins. Retrieved Ti particles from interfacial membranes of failed human THRs have been demonstrated to be endotoxin-free [3] and the particles have been shown not to induce an inflammatory response when exposed to macrophages *in vitro*.

The pathogenesis behind aseptic loosening is probably complex and chronic in nature. Important co-players are most likely implant instability [10;15;16;50;76], wear debris [85;91;138] and access and pressure of joint fluid [104;129;156;157] at bone-implant interface.

Migration of PE particles

In the cemented THAs fatigue cracks in the cemented mantle are believed to provide pathways for wear debris migrating in the implant-cement interface to the bone-cement interface leading to localized bone lesions [8;39]. Considering the problems with a cemented mechanical seal of the bone-implant interface, the possibility of a biological seal has been suggested [86]. We have defined a biological seal as a seal consisting of host tissue or other bio-active material, which has the potential of healing if defects occur due to mechanical stress. This way, a more durable seal theoretically can be created [120].

Only a few studies have compared the sealing effect of different orthopaedic surface coatings against particulate material. Bobyn et al [22] compared the sealing effect of a beaded porous implant surface with a smooth surface. The implants were exact fit inserted in the intracondylar notch or tibial plateau of a dog knee. Injections of PE particles were conducted 10 weeks after surgery and repeated twice a week for 10 weeks. After the last injections, a period of 10 weeks elapsed before euthanization of the animals. The smooth surface resulted in a fibrous membrane with the presence of PE particles, whereas the porous surface was osseo-integrated with no migration of PE particles.

Lalor et al [89] have used a similar model comparing polished Ti implants, HA implants and PMMA implants. Implants were press fit inserted in the condylar notch of a rabbit knee. Injections of PE particles were performed at 4 and 6 weeks postoperatively. At 8 weeks no differences in bone ongrowth were found and no qualitative differences in migration of PE particles were found. All implants had ongrowth of fibrous tissue in a small area adjacent to the joint space with accumulation of particles. Only trace amounts of particles were found around the implants more distant from the joint space.

Kraemer [86] et al compared the ability of smooth, porous coated, HA-coated and cemented hemiarthroplasties in canines to prevent migration of PE particles. Particles were only found in the sections from the smooth implants, which had ongrowth of fibrous tissue in all zones. The authors found equal sealing effect of HA-coated, porous-coated and cemented implants within the 5 months limit of the study, because no particles were detected around these implants.

The results on HA from these previous studies conflict in some degree with our data. We have proven that HA significantly improves the sealing effect of a grit-blasted or porous-coated Ti implant. These findings were not made by Lalor or Kraemer. These different results can be explained by the surgical fit, loading conditions and the particle load used in the experimental models.

When implants are press fit inserted in a stable and unloaded model like in the study of Lalor [89], optimal conditions for healing exist. However, these conditions are seldom present initially in the clinical situation. Optimal contact is never completely achieved [128]. Paul et al [118] found that a cementless femural stem press fit inserted in a broached femur was not in contact with bone tissue by an average of approximately 30 % and the average size of the gap was 0.9 mm in the proximal regions and even larger more distally. The gaps around implants must be regarded as

an extension of "the effective joint space" [130], and therefore as a potential pathway for migration of wear debris. Other studies have demonstrated that cementless components in the hip initially are subjected to micromotions in the range of 100-500 μm [26;158;158].

It is especially during these clinically relevant conditions with micromotion, weight-loading and poor surgical fit that HA has proven to provide the implant with superior bony anchorage and superior fixation [100;142;145-148]. It is therefore not surprising that no effect of HA was found in the study by Lalor with press fit inserted, unloaded and stable implants.

In the study by Kraemer [86], no difference in migration of particles was found around porous coated and hydroxyapatite coated implants, since no particles were found around these implants. Thus, an equal sealing effect was concluded for both coatings. In our study (III), we found a superior effect of HA as compared with porous coated implants. This might be explained by the different particle loads used in the two studies. To imitate the continuous production of wear debris in the clinical setting we injected the particles with short intervals. The load of particles was doubled as compared with the estimated load in a human hip joint after arthroplasty [97] and the dose was twenty-four times larger than the load used by Kraemer. The load was increased to put a maximal stress on the interface. Furthermore, injections of particles were conducted earlier after surgery in our study and repeated weekly instead of monthly. We hypothesized that by injecting the particles with small intervals between injections, the particles were allowed to accumulate in the interface in our study, because the threshold for the removal of particles by the lymphatic system was exceeded. In the study by Kraemer, particles may have been transported away from the interface by the lymphatic system before new particles entered due to the large intervals between injections and relatively low particle dose used. Thus, the difference in sealing effect between HA and porous coating was not detected.

The fibrous tissue surrounding a smooth or grit-blasted surface did not protect the interface against PE particles in our studies (I and II) or in the studies by Bobyn and Kraemer respectively. It has been speculated [89] that the marrow space may be effective in the clearance of particles by enabling the particles to be transported by the lymphatic system. Development of a fibrous tissue layer may inhibit particle removal through marrow spaces leading to entrapment and accumulation at the interface.

We believe that the ongrowth of bone is the main parameter determining the sealing effect of cementless implants. The effect of bony anchorage of implants on PE particle migration can be explained by two mechanisms. It acts as a mechanical barrier and it increases implant stability. By increasing the stability, the transport of wear particles by the pumping of fluid induced by an unstable interface can be avoided. From the present studies (I and II), we can conclude that the bonding of bone to the HA coating does not decrease with time, in contrast it increases. We reported 35 percent bone ingrowth after 8 weeks and 75 percent after 52 weeks.

These results might explain the clinical findings by Geesink et al [54], where radiological results of HA-coated primary total hip replacement have shown an increase in bone mass around the prosthesis, still improving 8 to 9 years after surgery. No cases with any mid or distal stem osteolysis were seen in this study, not even in cases with extensive PE wear. These results, along with results from D´Antonio et al [40], support the long-term sealing effect of HA coating.

The sealing effect of porous-surfaced implants without additional HA coating found by Kraemer and Bobyn can be explained, like the sealing effect of HA, by the osteo-conductive properties of the porous coating itself. This means that the porous structure serves as a passive scaffold, which enhances and supports bone ingrowth, which seals off the interface. However, another explanation on the sealing effect could be that the roughened surface of the implant influences the dynamics of joint fluid around the implants, thus providing a more stable environment in the

interface. This hypothesis can be supported by the data from tissue-ongrowth on Ti implants in study I and II. In these studies, we found no bone ongrowth to the implants after 8 weeks or after 52 weeks. The implants were surrounded by fibrous tissue. This is rather unexpected since the implants are stabilised by an anchorage screw and therefore conditions for healing are present. We believe that bone healing does not occur because the interface is stressed by the access of joint fluid to the interface. In study III, we found up till 32 % bone ingrowth around the porous-surfaced implants (5 out of 16 implants) after 16 weeks. Since the porous implant at the time of implantation was not in contact with the surrounding bone, it is difficult to explain the different healing response by the osteocondutive properties of the coating. This difference between grit blasted and porous coated implants in a gap situation could be due to a less fluid flow in the interface of porous surfaced implants encouraging bone to cross the gap.

Migration to distant organs

PE particles were found to migrate in huge amounts in the lymphatic system in agreement with clinical reports in study I and II [24;69], but were not detected in liver, spleen or lung tissue. However, this does not exclude the presence of submicron particles. Metal particles from patients with arthroplasties have been demonstrated by electron microscopy and mass spectrometry in tissues as bone marrow, spleen, liver, lung and kidney [31;90]. Widespread dissemination of wear debris should be taken seriously. Epidemiological studies have suggested a threefold risk for developing lymphoma and leukaemia for patients ten years after total joint replacement [159]. It has been suggested that the carcinogenic effect could be due to metallic wear [30]. However, more resent studies have not confirmed the reports of a significant increased risk of leukemia or lymphoma for patients, who have undergone metal on metal joint replacements and the overall cancer incidence of patients who have THA is slightly lower than in the general population [59;160].

Conclusion

The present studies demonstrate that hydroxyapatite applied on a grit-blasted or porous surface yields superior bony ingrowth, and provides an initial protection against the migration of polyethylene particles in the bone-implant interface compared with non-HA-coated implants. After 52 weeks the sealing effect of HA remains and the bone ongrowth is increased to the HA-coated implants.

PE particles did not induce inflammation or bone resorption in the bone-implant interface after 8 and 16 weeks. After 52 weeks, inflammation was seen around some particle-exposed Ti implants, however, this finding was not significant compared to control Ti implants. We find that the lack of adverse effect due to the particles could be explained by the endotoxin-free particles. Study III indicates that inflammation due to PE particles in the bone-implant interface may be a slow and time-dependent process.

Suggestions for future research

- The role of endotoxins in experimental and clinical particle-mediated osteolysis needs to be investigated. Experimental models studying the long-term effects of endotoxins in the bone-implant interface could be of interest. Until now, only data on the short-term effects exist [9].
- In the present studies only PE particles have been studied, however, studies including metallic, ceramic debris and smaller sized particles are needed. The composition and size of the particulate material may influence the osteolysis in peri-implant tissue.
- Wear debris are most likely produced in the artificial joint from the day the implant is inserted. It could therefore also be of interest to study the sealing effect of HA when particles are introduced in the joint or in the interface from day one after surgery.
- The knowledge about the sealing effect of different commercially available porous coatings is incomplete. We suggest that available coatings on the market should be compared in controlled experimental studies.
- We have in the present studies tested implant surfaces during stable conditions. However, it has been shown that micromotions occur in the bone-implant initially after the implantation of a cementless implant [26;158]. Studies on the sealing effect of surface coatings in models with controlled micromotions may therefore be more relevant.

References

1. Abu-Amer Y, Ross FP, Edwards J, Teitelbaum SL. Lipopolysaccharide-stimulated osteoclastogenesis is mediated by tumor necrosis factor via its P55 receptor. *J.Clin.Invest* 1997;100:1557-1565.
2. Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. *Endocrinology* 1998;139:663-670.
3. Akisue T, Bauer TW, Farver CF, Mochida Y. The effect of particle wear debris on NFkappaB activation and pro-inflammatory cytokine release in differentiated THP-1 cells. *J.Biomed.Mater.Res* 2002;59:507-515.
4. al Saffar N, Revell PA. Interleukin-1 production by activated macrophages surrounding loosened orthopaedic implants: a potential role in osteolysis. *Br.J.Rheumatol.* 1994;33:309-316.
5. Allen MJ, Brierley TJ, Millett PJ, Rushton N. The Effects of Particulate Polyethylene at a Weight-Bearing Bone-Implant Interface. A Study in Rats. *J.Bone Joint Surg.Br.* 1996;78-B:32-37.
6. Altman DG. *Practical statistics for medical research*. London: Chapman & Hall, 1991.
7. Amano S, Kawakami K, Iwahashi H, Kitano S, Hanazawa S. Functional role of endogenous CD14 in lipopolysaccharide-stimulated bone resorption. *J.Cell Physiol* 1997;173:301-309.
8. Anthony PP, Gie GA, Howie CR, Ling RS. Localised endosteal bone lysis in relation to the femoral components of cemented total hip arthroplasties. *J.Bone Joint Surg.Br.* 1990;72:971-979.
9. Aspenberg P. Adherent endotoxins are necessary for particle-induced bone resorption in a rat model. *Transaction Orthp Res Soc* 2000;25:704.
10. Aspenberg P, Herbertsson P. Periprosthetic bone resorption. Particles versus movement.*J.Bone Joint Surg.Br.* 1996;78:641-646.
11. Aspenberg P, Van Der Vis HM. Migration, particles, and fluid pressure. A discussion of causes of prosthetic loosening. *Clin.Orthop.* 1998;352;75-80.
12. Baddeley AJ, Gundersen HJ, Cruz-Orive LM. Estimation of surface area from vertical sections. *J.Microsc.* 1986;142:259-276.
13. Bauer TW, Geesink RC, Zimmerman R, McMahon JT. Hydroxyapatite-coated femoral stems. Histological analysis of components retrieved at autopsy. *J.Bone Joint Surg.Am.* 1991;73:1439-1452.
14. Bauer TW, Stulberg BN, Ming J, Geesink RG. Uncemented acetabular components. Histologic analysis of retrieved hydroxyapatite-coated and porous implants. *J.Arthroplasty* 1993;8:167-177.
15. Bechtold JE, Kubic V, Søballe K. A controlled experimental model of revision implants: Part I. Development. Acta Orthop.Scand. 2001;72:642-649.
16. Bechtold, J. E., Søballe, K., Kubic, V., Overgaard, S., Lewis, J. L., and Gustilo, R. B. Synergy between implant motion and particulate polyethylene in the formation of an aggressive periprosthetic membrane. *Transaction, ORS, 1997.*
17. Bellare A, Cohen RE. Morphology of rod stock and compression-moulded sheets of ultra-high-molecular-weight polyethylene used in orthopaedic implants. *Biomaterials* 1996;17:2325-2333.
18. Benz EB, Federman M, Godleski JJ, Bierbaum BE, Thornhill TS, Spector M. Transmission electron microscopy of intracellular particles of polyethylene from joint replacement prostheses: size distribution and cellular response. *Biomaterials* 2001;22:2835-2842.
19. Bi Y, Seabold JM, Kaar SG, Ragab AA, Goldberg VM, Anderson JM, Greenfiel EM. Adherent endotoxin on orthopedic wear particles stimulates cytokine production and osteoclast differentiation. *J.Bone Miner.Res* 2001;16:2082-2091.
20. Bloebaum RD, Beeks D, Dorr LD, Savory CG, DuPont JA, Hofmann AA. Complications with hydroxyapatite particulate separation in total hip arthroplasty. *Clin.Orthop.* 1994;298:19-26.
21. Bloebaum RD, Zou L, Bachus KN, Shea KG, Hofmann AA, Dunn HK. Analysis of particles in acetabular components from patients with osteolysis. *Clin.Orthop.* 1997;338:109-118.
22. Bobyn JD, Jacobs JJ, Tanzer M, Urban RM, Aribindi R, Sumner DR, Turner TM, Brooks CE. The susceptibility of smooth implant surfaces to periimplant fibrosis and migration of polyethylene wear debris. *Clin.Orthop.* 1995;311:21-39.
23. 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.* 1980;150;263-270.
24. Bos I, Johannisson R, Lohrs U, Lindner B, Seydel U. Comparative investigations of regional lymph nodes and pseudocapsules after implantation of joint endoprostheses. *Pathol.Res.Pract.* 1990;186:707-716.
25. Brooks RA, Sharpe JR, Wimhurst JA, Myer BJ, Dawes EN, Rushton N. The effects of the concentration of high-density polyethylene particles on the bone-implant interface. *J.Bone Joint Surg.Br.* 2000;82:595-600.

26. Burke DW, O'Connor DO, Zalenski EB, Jasty M, Harris WH. Micromotion of cemented and uncemented femoral components. *J.Bone Joint Surg.Br.* 1991;73:33-37.
27. Campbell P, Ma S, Yeom B, McKellop H, Schmalzried TP, Amstutz HC. Isolation of predominantly submicron-sized UHMWPE wear particles from periprosthetic tissues. *J.Biomed.Mater.Res.* 1995;29:127-131.
28. Capello WN. Hydroxyapatite in total hip arthroplasty: five-year clinical experience. *Orthopedics* 1994;17: 781, 792.
29. Capello WN, D'Antonio JA, Feinberg JR, Manley MT. Hydroxyapatite-coated total hip femoral components in patients less than fifty years old. Clinical and radiographic results after five to eight years of follow-up. *J.Bone Joint Surg.Am.* 1997;79: 1023-1029.
30. Case CP, Langkamer VG, Howell RT, Webb J, Standen G, Palmer M, Kemp A, Learmonth ID. Preliminary observations on possible premalignant changes in bone marrow adjacent to worn total hip arthroplasty implants. *Clin.Orthop.* 1996;329 Suppl: 269-279.
31. Case CP, Langkamer VG, James C, Palmer MR, Kemp AJ, Heap PF, Solomon L. Widespread dissemination of metal debris from implants [see comments]. *J.Bone Joint Surg.Br.* 1994;5:701-712.
32. Case CP, Langkamer VG, Lock RJ, Perry MJ, Palmer MR, Kemp AJ. Changes in the proportions of peripheral blood lymphocytes in patients with worn implants. *J.Bone Joint Surg.Br.* 2000;82:748-754.
33. Chiba J, Rubash HE, Kim KJ, Iwaki Y. The characterization of cytokines in the interface tissue obtained from failed cementless total hip arthroplasty with and without femoral osteolysis. *Clin.Orthop.* 1994;304-312.
34. Childs LM, Paschalis EP, Xing L, Dougall WC, Anderson D, Boskey AL, Puzas JE, Rosier RN, O'Keefe RJ, Boyce BF, Schwarz EM. In vivo RANK signaling blockade using the receptor activator of NF- kappaB:Fc effectively prevents and ameliorates wear debris-induced osteolysis via osteoclast depletion without inhibiting osteogenesis *J.Bone Miner.Res* 2002;17:192-199.
35. Ciccotti MG, Rothman RH, Hozack WJ, Moriarty L. Clinical and roentgenographic evaluation of hydroxyapatite-augmented and nonaugmented porous total hip arthroplasty. *J.Arthroplasty* 1994;9: 631-639.
36. Coathup MJ, Blunn GW, Flynn N, Williams C, Thomas NP. A comparison of bone remodelling around hydroxyapatite-coated, porous-coated and grit-blasted hip replacements retrieved at post-mortem. *J.Bone Joint Surg.Br.* 2001;83:118-123.
37. Collier JP, Bauer TW, Bloebaum RD, Bobyn JD, Cook SD, Galante JO, Harris WH, Head WC, Jasty MJ, Mayor MB, . Results of implant retrieval from postmortem specimens in patients with well-functioning, long-term total hip replacement. *Clin.Orthop.* 1992;97-112.
38. Cook SD, Thomas KA, Kay JF, Jarcho M. Hydroxyapatite-coated porous titanium for use as an orthopedic biologic attachment system. *Clin.Orthop.* 1988;230;303-312.
39. Crawford, R. W., Evans, E., Ling, R. S., and Murray, D. W. Fluid flow around model femoral components of differing surface finishes. Acta Orthop.Scand. 70(6), 589-595. 1999.
40. D'Antonio JA, Capello WN, Manley MT. Remodeling of bone around hydroxyapatite-coated femoral stems. *J.Bone Joint Surg.Am.* 1996;78: 1226-1234.
41. Daniels AU, Barnes FH, Charlebois SJ, Smith RA. Macrophage cytokine response to particles and lipopolysaccharide in vitro. *J.Biomed.Mater.Res* 2000;49:469-478.
42. de Bruijn JD, Flach JS, de Groot K, van Blitterswijk CA, Davies JE. Analysis of the bony interface with various types of hydroxyapatite in vitro. *Cells and materials* 1993;3:115-127.
43. de Groot K, Geesink R, Klein CP, Serekian P. Plasma sprayed coatings of hydroxylapatite. *J.Biomed.Mater.Res.* 1987;21:1375-1381.
44. Devane PA, Robinson EJ, Bourne RB, Rorabeck CH, Nayak NN, Horne JG. Measurement of polyethylene wear in acetabular components inserted with and without cement. A randomized trial [see comments]. *J.Bone Joint Surg.Am.* 1997;79:682-689.
45. Dorr LD, Wan Z, Song M, Ranawat A. Bilateral total hip arthroplasty comparing hydroxyapatite coating to porous-coated fixation. *J.Arthroplasty* 1998;13: 729-736.
46. Dowd JE, Schwendeman LJ, Macaulay W, Doyle JS, Shanbhag AS, Wilson S, Herndon JH, Rubash HE. Aseptic loosening in uncemented total hip arthroplasty in a canine model. *Clin.Orthop.* 1995;106-121.
47. Einhorn TA. Enhancement of fracture healing. *Instr.Course Lect.* 1996;45:401-416.
48. Emerson RHJ, Sanders SB, Head WC, Higgins L. Effect of circumferential plasma-spray porous coating on the rate of femoral osteolysis after total hip arthroplasty. *J.Bone Joint Surg.Am.* 1999;81: 1291-1298.
49. Ferrier GM, McEvoy A, Evans CE, Andrew JG. The effect of cyclic pressure on human monocyte-derived macrophages in vitro. *J.Bone Joint Surg.Br.* 2000;82:755-759.
50. Freeman MA, Plante-Bordeneuve P. Early migration and late aseptic failure of proximal femoral

prostheses. *J.Bone Joint Surg.Br.* 1994;76:432-438.
51. Frøkjær J, Deleuran B, Lind M, Overgaard S, Søballe K, Bünger C. Polyethylene particles stimulate monocyte chemotactic and activating factor production in synovial mononuclear cells in vivo. An immunohistochemical study in rabbits. *Acta Orthop.Scand.* 1995;66:303-307.
52. Frøkjær, J., Overgaard, S., Lind, M., Bunger, C., and Søballe, K. Failure of migration by injected polyethylene particles around press fit implants: and experimental study in rabbits. Hip International 9(4), 173-177. 1999.
53. Furlong RJ, Osborn JF. Fixation of hip prostheses by hydroxyapatite ceramic coatings. *J.Bone Joint Surg.Br.* 1991;73:741-745.
54. Geesink R, Hoefnagels N. Eight years results of HA-coated primary total hip replacement. *Acta Orthop.Belg.* 1997;63 Suppl 1:72-5:72-75.
55. Geesink RG. Hydroxyapatite-coated total hip prostheses. Two-year clinical and roentgenographic results of 100 cases. *Clin.Orthop.* 1990;261;39-58.
56. Geesink RG, de Groot K, Klein CP. Chemical implant fixation using hydroxyl-apatite coatings. The development of a human total hip prosthesis for chemical fixation to bone using hydroxyl-apatite coatings on titanium substrates. *Clin.Orthop.* 1987;225;147-170.
57. Geesink RG, Hoefnagels NH. Six-year results of hydroxyapatite-coated total hip replacement. *J.Bone Joint Surg.Br.* 1995;77:534-547.
58. Gelb H, Schumacher HR, Cuckler J, Ducheyne P, Baker DG. In vivo inflammatory response to polymethylmethacrylate particulate debris: effect of size, morphology, and surface area. *J.Orthop.Res* 1994;12:83-92.
59. Gillespie WJ, Henry DA, O'Connell DL, Kendrick S, Juszczak E, McInneny K, Derby L. Development of hematopoietic cancers after implantation of total joint replacement. *Clin.Orthop.* 1996;329 Suppl: 290-296.
60. Goldring SR, Jasty M, Roelke MS, Rourke CM, Bringhurst FR, Harris WH. Formation of a synovial-like membrane at the bone-cement interface. Its role in bone resorption and implant loosening after total hip replacement. *Arthritis Rheum.* 1986;29:836-842.
61. Goldring SR, Schiller AL, Roelke M, Rourke CM, O'Neil DA, Harris WH. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. *J.Bone Joint Surg.Am.* 1983;65:575-584.
62. Gonzalez O, Smith RL, Goodman SB. Effect of size, concentration, surface area, and volume of polymethylmethacrylate particles on human macrophages in vitro. *J.Biomed.Mater.Res* 1996;30: 463-473.
63. Gotfredsen K, Budtz-Jorgensen E, Jensen LN. A method for preparing and staining histological sections containing titanium implants for light microscopy. *Stain Technol.* 1989;64:121-127.
64. Green TR, Fisher J, Stone M, Wroblewski BM, Ingham E. Polyethylene particles of a 'critical size' are necessary for the induction of cytokines by macrophages in vitro. *Biomaterials* 1998;19:2297-2302.
65. Gross KA, Berndt CC, Goldschlag DD, Iacono VJ. In vitro changes of hydroxyapatite coatings. *Int.J.Oral Maxillofac.Implants.* 1997;12:589-597.
66. Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. .*APMIS* 1988;96:379-394.
67. Guttmann, D., Schmalzried, T. P., Jasty, M., and Harris, W. H. Light microscopic identification of submicron polyethylene wear debris. J.Appl.Biomater. 4, 303-307. 3-8-1993.
68. Havelin LI, Engesaeter LB, Espehaug B, Furnes O, Lie SA, Vollset SE. The Norwegian Arthroplasty Register: 11 years and 73,000 arthroplasties. *Acta Orthop.Scand.* 2000;71:337-353.
69. Hicks DG, Judkins AR, Sickel JZ, Rosier RN, Puzas JE, O'Keefe RJ. Granular histiocytosis of pelvic lymph nodes following total hip arthroplasty. The presence of wear debris, cytokine production, and immunologically activated macrophages [see comments]. *J.Bone Joint Surg.Am.* 1996;78:482-496.
70. Hirakawa K, Bauer TW, Hashimoto Y, Stulberg BN, Wilde AH, Secic M. Effect of femoral head diameter on tissue concentration of wear debris. *J.Biomed.Mater.Res.* 1997;36:529-535.
71. Howie DW, Vernon Roberts B, Oakeshott R, Manthey B. A rat model of resorption of bone at the cement-bone interface in the presence of polyethylene wear particles. *J.Bone Joint Surg.Am.* 1988;70:257-263.
72. Howling GI, Barnett PI, Tipper JL, Stone MH, Fisher J, Ingham E. Quantitative characterization of polyethylene debris isolated from periprosthetic tissue in early failure knee implants and early and late failure Charnley hip implants. *J.Biomed.Mater.Res.* 2001;58:415-420.
73. Imai S, Konttinen YT, Jumppanen M, Lindy O, Ceponis A, Kemppinen P, Sorsa T, Santavirta S, Xu JW, Lopez-Otin C. High levels of expression of collagenase-3 (MMP-13) in pathological conditions associated with a foreign-body reaction. *J.Bone Joint Surg.Br.* 1998;80:701-710.
74. Kadoya Y, Revell PA, al-Saffar N, Kobayashi A, Scott G, Freeman MA. Bone formation and

bone resorption in failed total joint arthroplasties: histomorphometric analysis with histochemical and immunohistochemical technique. *J.Orthop.Res.* 1996;14:473-482.

75. Kamikawa K, Harada Y, Nagata K, Moriya H. Differential effects of oxidised and non-oxidised polyethylene particles on human monocyte/ macrophages in vitro. *J.Bone Joint Surg.Br.* 2001;83:593-597.
76. Karrholm J, Borssen B, Lowenhielm 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;76:912-917.
77. Karrholm J, Frech W, Nivbrant B, Malchau H, Snorrason F, Herberts P. Fixation and metal release from the Tifit femoral stem prosthesis. 5-year follow-up of 64 cases. *Acta Orthop.Scand.* 1998;69: 369-378.
78. Karrholm J, Malchau H, Snorrason F, Herberts P, Rorabeck CH, Bourne RB, Laupacis A, Feeny D, Wong C, Tugwell P, Leslie K, Bullas R. Micromotion of femoral stems in total hip arthroplasty. A randomized study of cemented, hydroxyapatite-coated, and porous- coated stems with roentgen stereophotogrammetric analysis. *J.Bone Joint Surg.Am.* 1994;76:156-164.
79. Kay JF, Golec TS, Riley RL. Hydroxyapatite-coated subperiosteal dental implants: design rationale and clinical experience. *J.Prosthet.Dent.* 1987;58:339-343.
80. Kienapfel, H., Nilsson K, and Karrholm, J. Analysis of micromotion of porous-coated tibial total knee arthroplasty components. The effect of additional hydroxyapatite coating - a randomized RSA study. Transaction EORS 5, 54. 1995.
81. Kim KJ, Chiba J, Rubash HE. In vivo and in vitro analysis of membranes from hip prostheses inserted without cement. *J.Bone Joint Surg.Am.* 1994;76: 172-180.
82. Kim KJ, Kobayashi Y, Itoh T. Osteolysis model with continuous infusion of polyethylene particles. *Clin.Orthop.* 1998;46-52.
83. Kimmel DB, Jee WS. A quantitative histologic study of bone turnover in young adult beagles. *Anat.Rec.* 1982;203:31-45.
84. Knutson K, Lewold S, Robertsson O, Lidgren L. The Swedish knee arthroplasty register. A nation-wide study of 30,003 knees 1976-1992. *Acta Orthop.Scand.* 1994;65:375-386.
85. Kobayashi A, Freeman MA, Bonfield W, Kadoya Y, Yamac T, al Saffar N, Scott G, Revell PA. Number of polyethylene particles and osteolysis in total joint replacements. A quantitative study using a tissue-digestion method. *J.Bone Joint Surg.Br.* 1997;79: 844-848.
86. Kraemer WJ, Maistrelli GL, Fornasier V, Binnington A, Zhao JF. Migration of polyethylene wear debris in hip arthroplasties: a canine model. *J.Appl.Biomater.* 1995;6:225-230.
87. Kroon PO, Freeman MA. Hydroxyapatite coating of hip prostheses. Effect on migration into the femur. *J.Bone Joint Surg.Br.* 1992;74:518-522.
88. Kurtz SM, Muratoglu OK, Evans M, Edidin AA. Advances in the processing, sterilization, and crosslinking of ultra-high molecular weight polyethylene for total joint arthroplasty. *Biomaterials* 1999;20:1659-1688.
89. Lalor PA, Namba R, Mitchell SL, Bearcroff J, Beals N, Sledge CB, Spector M. Migration of polyethylene particles around stable implants in an animal model. *J.Long.Term.Eff.Med.Implants.* 1999;9:261-272.
90. Langkamer VG, Case CP, Heap P, Taylor A, Collins C, Pearse M, Solomon L. Systemic distribution of wear debris after hip replacement. A cause for concern? *J.Bone Joint Surg.Br.* 1992;74:831-839.
91. Learmonth ID, Hussell JG, Smith EJ. Inadequate polyethylene thickness and osteolysis in cementless hip arthroplasty. *J.Arthroplasty* 1997;12:305-309.
92. LeGeros P, Orly I, Gregoire M, Daculsi G. Substrate surface dissolution and interfacial biological mineralization. In: Davies JE, ed. The bone-biomaterial interface. Toronto: University of Toronto Press, 1991:76-88.
93. Loupasis G, Morris EW, Hyde ID. The Furlong hydroxyapatite-coated total hip replacement in patients under age 51. A 6-year follow-up study. *Acta Orthop.Belg.* 1998;64:17-24.
94. Lucht U. The Danish Hip Arthroplasty Register. *Acta Orthop.Scand.* 2000;71:433-439.
95. Malchau H, Herberts P, Söderman P, Odén A. Prognosis of Total Hip Replacement. Update and Validation of Results form the Swedish National Arthroplasty Hip Register 1979-98. *Scientific Exhibition presented at 67th Annual Meeting of the American Association of Orthopaedic Surgeons* 2000.
96. Maxian SH, Zawadsky JP, Dunn MG. In vitro evaluation of amorphous calcium phosphate and poorly crystallized hydroxyapatite coatings on titanium implants. *J.Biomed.Mater.Res.* 1993;27: 111-117.
97. McKellop HA, Campbell P, Park SH, Schmalzried TP, Grigoris P, Amstutz HC, Sarmiento A. The Origin of Submicron Polyethylene Wear Debris in Total Hip Arthroplasty. *Clin.Orthop.* 1995;311:3-20.
98. McNally SA, Shepperd JA, Mann CV, Walczak JP. The results at nine to twelve years of the use of a hydroxyapatite-coated femoral stem. *J.Bone Joint Surg.Br.* 2000;82:378-382.
99. Mirra JM, Amstutz HC, Matos M, Gold R. The

pathology of the joint tissues and its clinical relevance in prosthesis failure. *Clin.Orthop.* 1976;117:221-240.

100. Mouzin O, Søballe K, Bechtold JE. Loading improves anchorage of hydroxyapatite implants more than titanium implants. *J.Biomed.Mater.Res.* 2001;58:61-68.
101. Murray DW, Rushton N. Macrophages stimulate bone resorption when they phagocytose particles. *J.Bone Joint Surg.Br.* 1990;72:988-992.
102. Nakashima Y, Sun DH, Trindade MC, Chun LE, Song Y, Goodman SB, Schurman DJ, Maloney WJ, Smith RL. Induction of macrophage C-C chemokine expression by titanium alloy and bone cement particles. *J.Bone Joint Surg.Br.* 1999;81:155-162.
103. Nelissen RG, Valstar ER, Rozing PM. The effect of hydroxyapatite on the micromotion of total knee prostheses. A prospective, randomized, double-blind study. *J.Bone Joint Surg.Am.* 1998;80:1665-1672.
104. Nivbrant B, Karlsson K, Karrholm J. Cytokine levels in synovial fluid from hips with well-functioning or loose prostheses. *J.Bone Joint Surg.Br.* 1999;81: 163-166.
105. Nordsletten L, Hogasen AK, Konttinen YT, Santavirta S, Aspenberg P, Aasen AO. Human monocytes stimulation by particles of hydroxyapatite, silicon carbide and diamond: in vitro studies of new prosthesis coatings. *Biomaterials* 1996;17:1521-1527.
106. Ohlin A, Lerner UH. Bone-resorbing activity of different periprosthetic tissues in aseptic loosening of total hip arthroplasty. *Bone Miner.* 1993;20:67-78.
107. Onsten I, Carlsson AS, Sanzen L, Besjakov J. Migration and wear of a hydroxyapatite-coated hip prosthesis. A controlled roentgen stereophotogrammetric study. .*J.Bone Joint Surg.Br.* 1996;78:85-91.
108. Onsten I, Nordqvist A, Carlsson AS, Besjakov J, Shott S. Hydroxyapatite augmentation of the porous coating improves fixation of tibial components. A randomised RSA study in 116 patients. *J.Bone Joint Surg.Br.* 1998;80:417-425.
109. Oonishi H, Kadoya Y. Wear of high-dose gamma-irradiated polyethylene in total hip replacements. *J.Orthop.Sci.* 2000;5:223-228.
110. Overgaard S. Calcium phosphate coatings for fixation of implants. *Acta Orthop.Scand.Suppl.279* 2000;71:1-74.
111. Overgaard S, Bromose U, Lind M, Bunger C, Søballe K. The influence of crystallinity of the hydroxyapatite coating on the fixation of implants. Mechanical and histomorphometric results. *J.Bone Joint Surg.Br.* 1999;81:725-731.
112. Overgaard S, Knudsen HM, Hansen LN, Mossing N. Hip arthroplasty in Jutland, Denmark. Age and sex-specific incidences of primary operations. *Acta Orthop.Scand.* 1992;63:536-538.
113. Overgaard S, Lind M, Glerup H, Bunger C, Søballe K. Porous-coated versus grit-blasted surface texture of hydroxyapatite-coated implants during controlled micromotion: mechanical and histomorphometric results. *J.Arthroplasty* 1998;13:449-458.
114. Overgaard S, Lind M, Glerup H, Grundvig S, Bünger C, Søballe K. Hydroxyapatite and fluorapatite coatings for fixation of weight loaded implants. *Clin.Orthop.* 1997;336;286-296.
115. Overgaard S, Søballe K, Jorgen H, Gundersen G. Efficiency of systematic sampling in histomorphometric bone research illustrated by hydroxyapatite-coated implants: optimizing the stereological vertical-section design. *J.Orthop.Res.* 2000;18:313-321.
116. Overgaard S, Søballe K, Josephsen K, Hansen ES, Bünger C. Role of different loading conditions on resorption of hydroxyapatite coating evaluated by histomorphometric and stereological methods. *J.Orthop.Res.* 1996;14:888-894.
117. Pap T, Pap G, Hummel KM, Franz JK, Jeisy E, Sainsbury I, Gay RE, Billingham M, Neumann W, Gay S. Membrane-type-1 matrix metalloproteinase is abundantly expressed in fibroblasts and osteoclasts at the bone-implant interface of aseptically loosened joint arthroplasties in situ. *J.Rheumatol.* 1999;26: 166-169.
118. Paul HA, Bargar WL, Mittlestadt B, Musits B, Taylor RH, Kazanzides P, Zuhars J, Williamson B, Hanson W. Development of a surgical robot for cementless total hip arthroplasty. *Clin.Orthop.* 1992;285;57-66.
119. Ragab AA, Van De Motter R, Lavish SA, Goldberg VM, Ninomiya JT, Carlin CR, Greenfield EM. Measurement and removal of adherent endotoxin from titanium particles and implant surfaces. *J.Orthop.Res.* 1999;17:803-809.
120. Rahbek O, Overgaard S, Jensen TB, Bendix K, Søballe K. Sealing effect of hydroxyapatite coating: a 12-month study in canines. *Acta Orthop.Scand.* 2000;71:563-573.
121. Rahbek O, Overgaard S, Kold S, Søballe K. The Effect of Particulate Hydroxyapatite and Polyethylene on Mechanical Fixation of Hydroxyapatite Coated Implants. *Transaction EORS* ;June 1-3, 2001:40.
122. Rokkum M, Brandt M, Bye K, Hetland KR, Waage S, Reigstad A. Polyethylene wear, osteolysis and acetabular loosening with an HA-coated hip prosthesis. A follow-up of 94 consecutive arthroplasties [see comments]. *J.Bone Joint Surg.Br.* 1999;81:582-589.
123. Rossi P, Sibelli P, Fumero S, Crua E. Short-term results of hydroxyapatite-coated primary total hip arthroplasty. *Clin.Orthop.* 1995;310;98-102.
124. Rothman RH, Hozack WJ, Ranawat A, Moriarty L.

Hydroxyapatite-coated femoral stems. A matched-pair analysis of coated and uncoated implants. *J.Bone Joint Surg.Am.* 1996;78:319-324.

125. Santavirta S, Hoikka V, Eskola A, Konttinen YT, Paavilainen T, Tallroth K. Aggressive granulomatous lesions in cementless total hip arthroplasty. *J.Bone Joint Surg.Br.* 1990;72:980-984.
126. Santavirta S, Konttinen YT, Bergroth V, Eskola A, Tallroth K, Lindholm TS. Aggressive granulomatous lesions associated with hip arthroplasty. Immunopathological studies. *J.Bone Joint Surg.Am.* 1990;72:252-258.
127. Sasaki T, Watanabe C. Stimulation of osteoinduction in bone wound healing by high-molecular hyaluronic acid. *Bone* 1995;16:9-15.
128. Schimmel JW, Huiskes R. Primary fit of the Lord cementless total hip. A geometric study in cadavers. *Acta Orthop.Scand.* 1988;59:638-642.
129. Schmalzried TP, Akizuki KH, Fedenko AN, Mirra J. The role of access of joint fluid to bone in periarticular osteolysis. A report of four cases [see comments]. *J.Bone Joint Surg.Am.* 1997;79:447-452.
130. Schmalzried TP, Jasty M, Harris WH. Periprosthetic bone loss in total hip arthroplasty. Polyethylene wear debris and the concept of the effective joint space. *J.Bone Joint Surg.Am.* 1992;74:849-863.
131. Schmalzried TP, Jasty M, Rosenberg A, Harris WH. Histologic identification of polyethylene wear debris using Oil Red O stain. *J.Appl.Biomater.* 1993;4:119-125.
132. Schmalzried TP, Szuszczewicz ES, Northfield MR, Akizuki KH, Frankel RE, Belcher G, Amstutz HC. Quantitative assessment of walking activity after total hip or knee replacement. *J.Bone Joint Surg.Am.* 1998;80:54-59.
133. Shanbhag AS, Hasselman CT, Rubash HE. The John Charnley Award. Inhibition of wear debris mediated osteolysis in a canine total hip arthroplasty model. *Clin.Orthop.* 1997;33-43.
134. Shanbhag AS, Jacobs JJ, Black J, Galante JO, Glant TT. Macrophage/particle interactions: effect of size, composition and surface area. *J.Biomed.Mater.Res.* 1994;28:81-90.
135. Shanbhag AS, Jacobs JJ, Black J, Galante JO, Glant TT. Human monocyte response to particulate biomaterials generated in vivo and in vitro .*J.Orthop.Res.* 1995;13:792-801.
136. Shanbhag AS, Jacobs JJ, Glant TT, Gilbert JL, Black J, Galante JO. Composition and morphology of wear debris in failed uncemented total hip replacement. *J.Bone Joint Surg.Br.* 1994;76:60-67.
137. Shimizu C, Yoshioka M, Coutts RD, Harwood FL, Kubo T, Hirasawa Y, Amiel D. Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. *Osteoarthritis.Cartilage.* 1998;6:1-9.
138. Sochart DH. Relationship of acetabular wear to osteolysis and loosening in total hip arthroplasty. *Clin.Orthop.* 1999;135-150.
139. Spector M, Shortkroff S, Hsu HP, Taylor-Zapatka S, Lane N, Sledge CB, Thornhill TS. Synovium-like tissue from loose joint replacement prosthesis: comparison of human material with a canine model. *Semin.Arthritis Rheum.* 1992;21:335-344.
140. Szponar B, Larsson L. Use of mass spectrometry for characterising microbial communities in bioaerosols. *Ann.Agric.Environ.Med.* 2001;8:111-117.
141. Søballe K. Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs. *Acta Orthop.Scand.Suppl.* 1993;255:1-58.
142. Søballe K, Brockstedt Rasmussen H, Hansen ES, Bunger C. Hydroxyapatite coating modifies implant membrane formation. Controlled micromotion studied in dogs. *Acta Orthop.Scand.* 1992;63:128-140.
143. Søballe K, Gotfredsen K, Brockstedt Rasmussen H, Nielsen PT, Rechnagel K. Histologic analysis of a retrieved hydroxyapatite-coated femoral prosthesis. *Clin.Orthop.* 1991;272;255-258.
144. Søballe K, Hansen ES, Brockstedt-Rasmussen H, Pedersen CM, Bunger C. Bone graft incorporation around titanium-alloy and hydroxyapatite-coated implants in dogs. *Clin.Orthop.* 1992;274;282-293.
145. Søballe K, Hansen ES, Brockstedt Rasmussen H, Bunger C. Hydroxyapatite coating converts fibrous tissue to bone around loaded implants. *J.Bone Joint Surg.Br.* 1993;75:270-278.
146. Søballe K, Hansen ES, Brockstedt Rasmussen H, Hjortdal VE, Juhl GI, Pedersen CM, Hvid I, Bunger C. Gap healing enhanced by hydroxyapatite coating in dogs. *Clin.Orthop.* 1991;272:300-307.
147. Søballe K, Hansen ES, Brockstedt Rasmussen H, Pedersen CM, Bunger C. Hydroxyapatite coating enhances fixation of porous coated implants. A comparison in dogs between press fit and noninterference fit. *Acta Orthop.Scand.* 1990;61: 299-306.
148. Søballe K, Hansen ES, Rasmussen HB, Jorgensen PH, Bünger C. Tissue ingrowth into titanium and hydroxyapatite-coated implants during stable and unstable mechanical conditions. *J.Orthop.Res.* 1992;10:285-299.
149. Søballe K, Toksvig Larsen S, Gelineck J, Fruensgaard S, Hansen ES, Ryd L, Lucht U, Bünger C. Migration of hydroxyapatite coated femoral prostheses. A Roentgen Stereophotogrammetric study. *J.Bone Joint Surg.Br.* 1993;75:681-687.
150. Tabata Y, Ikada Y. Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. *Biomaterials* 1988;9: 356-362.
151. Thanner J, Karrholm J, Herberts P, Malchau H.

Porous cups with and without hydroxylapatite-tricalcium phosphate coating: 23 matched pairs evaluated with radiostereometry. *J.Arthroplasty* 1999;14:266-271.

152. Tonino AJ, Romanini L, Rossi P, Borroni M, Greco F, Garcia-Araujo C, Garcia-Dihinx L, Murcia-Mazon A, Hein W, Anderson J. Hydroxyapatite-coated hip prostheses. Early results from an international study. *Clin.Orthop.* 1995;211-225.
153. Tonino AJ, Therin M, Doyle C. Hydroxyapatite-coated femoral stems. Histology and histomorphometry around five components retrieved at post mortem. *J.Bone Joint Surg.Br.* 1999;81:148-154.
154. Urban RM, Jacobs JJ, Tomlinson MJ, Gavrilovic J, Black J, Peoc'h M. Dissemination of wear particles to the liver, spleen, and abdominal lymph nodes of patients with hip or knee replacement [see comments]. *J.Bone Joint Surg.Am.* 2000;82:457-476.
155. Van Der Vis HM, Marti RK, Tigchelaar W, Schuller HM, Van Noorden CJ. Benign cellular responses in rays to different wear particles in intraarticular and intramedullary environment. *J.Bone Joint Surg.Br.* 1997;79:837-843.
156. Van Der Vis HM, Aspenberg P, de Kleine R, Tigchelaar W, Van Noorden CJ. Short periods of oscillating fluid pressure directed at a titanium-bone interface in rabbits lead to bone lysis. *Acta Orthop.Scand.* 1998;69:5-10.
157. Van Der Vis HM, Aspenberg P, Marti RK, Tigchelaar W, Van-Noorden CJ. Fluid pressure causes bone resorption in a rabbit model of prosthetic loosening. *Clin.Orthop.* 1998;350;201-208.
158. Vanderby R, Jr., Manley PA, Kohles SS, McBeath AA. Fixation stability of femoral components in a canine hip replacement model. *J.Orthop.Res.* 1992;10:300-309.
159. Visuri T, Koskenvuo M. Cancer risk after Mckee-Farrar total hip replacement. *Orthopedics.* 1991;14: 137-142.
160. Visuri T, Pukkala E, Paavolainen P, Pulkkinen P, Riska EB. Cancer risk after metal on metal and polyethylene on metal total hip arthroplasty. *Clin.Orthop.* 1996;329 suppl.;280-289.
161. Wenz W, Breusch SJ, Graf J, Stratmann U. Ultrastructural findings after intraarticular application of hyaluronan in a canine model of arthropathy. *J.Orthop.Res* 2000;18:604-612.
162. Whitehouse WJ. The quantitative morphology of anisotropic trabecular bone. *J.Microsc.* 1974;101: 153-168.
163. Wiliams, D. F. Definitions in biomaterials. 1-72. 1987. Amsterdam, Elsevier Publishers.
164. Wiliams, D. F., Black, J., and Doherty, P. J. Second consensus conference on definitions in biomaterials. Doherty, P. J. 525-533. 1992. London, Elsevier Publishers. Biomaterials-tissue interfaces; Advances in biomaterials.
165. Willert HG. Reactions of the articular capsule to wear products of artificial joint prostheses. *J.Biomed.Mater.Res.* 1977;11:157-164.
166. Wirta J, Eskola A, Santavirta S, Tallroth K, Konttinen YT, Lindholm S. Revision of aggressive granulomatous lesions in hip arthroplasty. *J.Arthroplasty* 1990;5 Suppl:S47-S52.
167. Wooley PH, Nasser S, Fitzgerald RH, Jr. The immune response to implant materials in humans.*Clin.Orthop.* 1996;63-70.
168. Wooley PH, Petersen S, Song Z, Nasser S. Cellular immune responses to orthopaedic implant materials following cemented total joint replacement. *J.Orthop.Res* 1997;15:874-880.
169. Yao J, Glant TT, Lark MW, Mikecz K, Jacobs JJ, Hutchinson NI, Hoerrner LA, Kuettner KE, Galante JO, Cs Szabo G. The potential role of fibroblasts in periprosthetic osteolysis: fibroblast response to titanium particles Suppression of osteoblast function by titanium particles. .*J.Bone Miner.Res.* 1997;79: 107-112.
170. Yee AJ, Kreder HK, Bookman I, Davey JR. A randomized trial of hydroxyapatite coated prostheses in total hip arthroplasty. *Clin.Orthop.* 1999;120-132.
171. Yoshimi T, Kikuchi T, Obara T, Yamaguchi T, Sakakibara Y, Itoh H, Iwata H, Miura T. Effects of high-molecular-weight sodium hyaluronate on experimental osteoarthrosis induced by the resection of rabbit anterior cruciate ligament. *Clin.Orthop.* 1994;298;296-304.
172. Zhang X, Morham SG, Langenbach R, Young DA, Xing L, Boyce BF, Puzas EJ, Rosier RN, O'Keefe RJ, Schwarz EM. Evidence for a direct role of cyclo-oxygenase 2 in implant wear debris-induced osteolysis. *J.Bone Miner.Res* 2001;16:660-670.