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Calcium phosphate coatings for fixation of bone implants

Evaluated mechanically and histologically by stereological methods

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THESIS

ACTA ORTHOPAEDICA SCANDINAVICA SUPPLEMENTUM NO. 297, VOL. 71, 2000

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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–VIII).

I 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(6): 888-894 [305]

II Overgaard S, Lind M, Rahbek O, Bünger C, Søballe K. Improved fixation of porous-coated versus grit-blasted surface texture of hydroxyapatite-coated implants in dogs. Acta Orthop Scand 1997; 68(4): 337-343 [302]

III Overgaard S, Lind M, Glerup H, Bünger 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(4): 449-458 [299]

IV 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. [300]

V Overgaard S, Lind M, Josephsen K, Maunsbach AB, Bünger C, Søballe K. Resorption of hydroxyapatite and fluorapatite ceramic coatings on weight-bearing implants. A quantitative and morphological study in dogs. J Biomed Mater Res 1998; 39(1): 141-152. [301]

VI Overgaard S, Søballe K, Lind M, Bünger C. Resorption of hydroxyapatite and fluorapatite coatings in man. An experimental study in trabecular bone. J Bone Joint Surg (Br) 1997; 79(4): 654-9 [306]

VII Overgaard S, Bromose U, Lind M, Bünger 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: 725-31[297]

VIII Overgaard S, Søballe K, Gundersen HJG. Efficiency of systematic sampling in histomorphometric bone research illustrated by hydroxyapatite-coated implants. Optimizing of the stereological vertical section design. J Orthop Res 2000; 18: 313-321[304]

Denne afhandling er i forbindelse med de nedenfor anførte tidligere publicerede afhandlinger af Det Sundhedsvidenskabelige Fakultet ved Aarhus Universitet, antaget til offentlig forsvar for den medicinske doktorgrad.

Forsvaret finder sted den 10. november 2000 kl 14.00 præcis i Anatomisk Instituts undervisningsfløj, auditorium 424, indgang 230, Aarhus Universitet.

Aarhus Universitet 19. juni 2000

Søren Mogensen
Dekan

Preface

The present thesis is based on investigations performed at the Orthopaedic Research Laboratory, Department of Orthopaedics, Aarhus University Hospital, Denmark during a research fellowship (grant by The Danish Rheumatism Association) in the period 1994–97. The investigations were performed at the following institutions: Orthopaedic Research Laboratory, Department of Orthopaedics, Aarhus University Hospital; Stereological Research Laboratory, Aarhus University Hospital; Institute of Experimental Clinical Research, University of Aarhus; Department of Dental Pathology, Royal Dental College, University of Aarhus; Institute of Pathology, Amtssygehuset, Aarhus University Hospital; Department of Earth Science, University of Aarhus; Department of Cell Biology, Institute of Anatomy, University of Aarhus.

The present series of studies was initiated by the work of associate professor Kjeld Søballe, M.D., D.M.Sc. to whom I am deeply indebted for introducing me to bone implant research and for inestimable support during the initial surgical procedures. I thank him for his invaluable advice and friendship throughout the studies and for providing a forum for stimulating discussions. I also wish to express my deepest gratitude to professor Cody Bünger, M.D., D.M.Sc. for introducing me to this field and to experimental orthopaedic research. I thank him for his invaluable support and advice and for his constructive criticism and friendship throughout the studies.

I am very grateful to professor Otto Sneppen, M.D., D.M.Sc. for valuable support and for providing excellent working conditions in the Orthopedic Research Laboratory and to professor Jens Christian Djurhuus, M.D., D.M.Sc. for the unique facilities at Institute of Experimental Clinical Research.

Faced with the problem of how to quantify, I met professor Hans Jørgen G. Gundersen, M.D., D.M.Sc., Stereological Research Laboratory. With great enthusiasm he taught me stereology. To him I express my greatest thanks. A special thank is directed to Martin Lind, M.D., Ph.D., D.M.Sc. for his excellent support during the experiments and for our challenging discussions at the office. I wish to thank associate professor Kaj Josephsen, D.D.S., Royal Dental College Aarhus, for his congenial cooperation and for providing excellent facilities at the EM laboratory, Royal Dental College. Without the outstanding effort of my other research collaborators Ulf Bromose, M.D., Henning Glerup M.D., Ph.D., associate professor Sidsel Grundvig, Ph.D., Ebbe Stender Hansen, M.D., D.M.Sc., professor Arvid B. Maunsbach, M.D., D.M.Sc., and Ole Rahbek M.D., these studies would not have been possible and I thank them for their excellent cooperation.

Special thanks to Anette Milton, Lis Lund, Jane Pauli, Merete Andersen, and Rita Ullerup for excellent technical assistance and for taking part in histological evaluation. Moreover, thanks to associate professor Knud Bendix, M.D., D.M.Sc., Institute of Pathology for the preparation and evaluation of synovial biopsies; professor Flemming Melsen, M.D., D.M.Sc., Institute of Pathology, for histomorphometric advice; associate professor Joop Wolke, Ph.D., University of Nijmegen, The Netherlands, for the evaluation of coating crystallinity; associate professor Morten Frydenberg, Department of Biostatistics, University of Aarhus for statistical advice; and thanks to Linda Nygaard, BA, AA, for linguistic advice.

But above all, I would like to express my love and thanks to my wife Charlotte, and our children Christine, Laura and Casper for their love, patience and support throughout the work.

Contents

Abstract

The osteoconductive properties of HA coatings are well-documented. HA coating is able to enhance bone ingrowth and to reduce early migration of both hip and knee prostheses. Despite the clinical use of HA-coated prostheses several aspects relevant to HA coatings have not been elucidated. The optimum coating quality and surface texture is still a matter of debate. Moreover, the significance of coating resorption is controversial. It has been suggested that resorption disintegrates the coating and reduces the bonding strength between implant and bone and the strength of the coating-implant interface, which might lead to implant loosening, coating delamination and acceleration of third body wear processes.

This thesis aimed to investigate the effects of Ca-P coating type, quality and surface texture on mechanical fixation, bone ingrowth and loss of coating in experimental models in dogs and man. Furthermore, the significance of systematic sampling in bone histomorphometry using the unbiased stereological vertical section method was analyzed.

Results. The first group of studies showed that HA-coated implants with porous-coated surface demonstrated increased energy absorption compared with grit-blasted implants during both non weight-bearing and weight-bearing conditions with controlled micromotion of 500 μm. In addition, the HA coating delaminated on grit-blasted implants during mechanical testing in contrast to porous-coated implants. Histomorphometry showed increased bone ingrowth to grit-blasted implants demonstrating that surface topology influenced surface activity.

The next series of studies focused on the effects of Ca-P coating type, HA versus FA, during stable weight-bearing and non weight-bearing conditions. In dogs, no difference in mechanical fixation and bone ingrowth was demonstrated. However, in humans, HA-coated implants had significantly greater bone ongrowth than FA-coated implants after one year.

The third group of studies evaluated the effects of HA coating crystallinity during controlled micromotion of 250 μm. After 16 weeks, low crystalline (50%) HA coating accelerated mechanical fixation and bone ingrowth compared with high crystalline HA (75%). High crystalline HA achieved significantly better anchorage from 16 to 32 weeks whereas mechanical fixation of low crystalline HA was unchanged.

In all studies, loss of Ca-P coating was evaluated. It was demonstrated that the coatings were resorbed, partially, in vivo irrespective type and quality of the coating. HA coverage on porous-coated implants was significantly more reduced than on grit-blasted implants in dogs. No difference in overall resorption between HA and FA coatings was demonstrated. However, in humans, significantly less HA and FA coating was resorbed when bone was present on the coating surface compared with bone marrow or fibrous tissue. In addition, resorption of HA was greater than FA in the presence of bone marrow indicating that FA was more stable than HA. Low (50%) crystalline HA coating was significantly more reduced compared with high (75%) crystalline HA at both 16 and 32 weeks. However, no further coating loss was observed from 16 to 32 weeks suggesting two phases of coating resorption: *Phase I (0–16 weeks)* with rapid coating loss, and *phase II (16–32 weeks)* with slow loss. Another important finding was that continuous loading and micromovements of 150 μm accelerated resorption in contrast to immobilization of the implant. In addition, unstable fibrous anchored implants had significantly more loss of HA coating as compared with bony anchored implants. In all studies, resorbed coating was partly replaced by bone in direct contact with the implant surface suggesting durable implant fixation.

Sampling efficiency in the unbiased stereological vertical section method was analyzed in order to find an optimal sampling design for histomorphometric analyzes at different sampling levels (humans, sections, fields of view and number of counting items) with different sampling intensi-

ties. The analysis showed that only minor changes in variances were observed when the initial scheme of 14 sections from each implant was reduced to include only one of the two possible implant sides, every third field of view and half the probe density, reducing the total workload at the microscope to less than 10% on all sections. In addition, the number of sections for analysis could be reduced to every fourth section per implant (3–4 sections for evaluation) without significantly increase in variance. The study demonstrated that biological variation contributed to the majority of the total observed variance.

Conclusion. The present series of investigations demonstrated that Ca-P coating type and quality and the underlying surface texture had significant influence on either mechanical fixation, bone in/ongrowth and loss of coating in dogs and man. In addition, the sampling design for histomorphometry could be optimized without reducing the quality of the data.

Definitions

Bioactive [#] — Bioactive materials are designed to elicit or modulate biological activity.

Biocompatibility [#] — The ability of a material used in a medical device to perform with an appropriate host response in a specific application.

Biomaterial [#] — Material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body.

Bone ingrowth or ongrowth — The terms bone ingrowth and ongrowth were used for porous-coated and a grit-blasted implants, respectively. In- or ongrowth was defined as direct contact between bone and implant surface (Ca-P coating or titanium-alloy) at the light microscopic or at the scanning electron microscopic level.

Ceramics — Any material of non-metal and inorganic origin.

Corrosion — Interaction between a metal and the environment resulting in release of ions and degradation of the material.

Delamination — Separation of a coating into layers or separation of the entire coating.

Dissolution — The process in which one substance is dissolved into another.

Foreign body reaction [#] — Variation in normal tissue behavior caused by the presence of a foreign material.

Histomorphometry — Quantitative evaluation of tissue dimensions.

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.

Implant fixation — A general term for mechanical and biological implant fixation.

Loss of coating — Several different terms are used for loss of ceramic coatings in the biological system; examples of such terms include degradation, biodegradation, resorption, bioresorption, break down, and gradual fragmentation [413,415]. In the present review loss of coating is used as a general term irrespective type of mechanism: cellular activity, simple dissolution or mechanical removal (wear, abrasion, delamination).

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 formation of bone at extraskeletal sites [107].

Resorption[#] — Reduction of coating because of cellular activity or simple dissolution.

Shear — Force or stress occurring under displacement of two parallel surfaces relative to each other.

Solubility — Susceptibility of being dissolved. Determined by the thermodynamic solubility product.

Stereology — The study of the three-dimensional properties of objects. Stereological methods are precise tools for obtaining quantitative information about three-dimensional structures based on observations made on two-dimensional sections [155].

Strain — Relative deformation of an object.

Stress — The force that develop within an object or its surface when external load is applied.

Tensile — Force or stress occurring when tension is applied to an object.

[#]Definitions agreed on consensus conferences on biomaterials from 1987 and 1992 [413,415].

Abbreviations

Ca	Calcium
Ca-P	Calcium phosphate
EM	Electron microscopy
FA	Fluorapatite
FDA	Food and drug administration in United States
HA	Hydroxyapatite
LM	Light microscopy
P	Phosphate
SEM	Scanning electron microscopy
SD	Standard deviation
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
TCP	Tricalciumphosphate
Ti-6Al-4V	Titanium-6aluminum-4vanadium
XRD	X-ray diffraction

Introduction

Clinical background

During the last decades artificial joints have been a successful treatment for primary and secondary osteoarthrosis particularly of hip, knee, and shoulder joints [260]. Approximately 8000 joint arthroplasties are implanted in Denmark yearly, the majority being primary THA corresponding to an overall incidence of 82 primary THA per 100,000 inhabitants per year [298]. The incidence in Sweden has varied between 101–141 per 100,000 inhabitants per year during the last five years, whereas the demand in the USA has been reported to be approximately 50 per 100,000 inhabitants [259,277]. In Denmark, the incidence of THA has been unchanged for several years. However, due to changes in demographics the total demand for THA will increase 33% during the next 20 years [191,298]. Economically, significant resources are expended on artificial joints, and costs of revision surgery are many times greater than those of primary operation [126,240,338].

In the early 1980s, bone cement was suspected to play a major role in bone resorption and aseptic loosening of prostheses which led to the introduction of prostheses for cementless use [139,169,211]. Currently, the cementing technique has been improved at several levels which seems to reduce revision rates [113,211,259,272]. Cement is the current standard for elderly patients with low activity level and a relatively short life expectancy. However, because of the inherent biological and mechanical properties of cement this fixation method might be insufficient for younger patients and non-cemented prostheses might be an alternative. The cementless technique relies on biological fixation provided by initial press fit insertion followed by bone ingrowth into a textured or porous implant surface [24,131,360].

The overall clinical results in large series of cemented THA and TKA are good, with a 5–10% risk of revision within 10 years after surgery. However, for younger patients the risk of implant failure and revision of the prosthesis is unsatisfactorily high: rates of 20–25 % have been published [235,260]. Few prospective randomized studies on cemented versus cementless prostheses have been published, however, the cementless technique has not proven to be better than the cemented [215,290,339]. Regarding cementless femoral components for THA it has been shown that thigh pain is a significant problem and that osteolysis around the implant may be present in up to 40% of cases within ten years after surgery [17,111,137]. In addition, retrieval studies have revealed that the majority of femoral stems and acetabular components become fixed by fibrous tissue instead of bone ingrowth [62,67,361]. Thus, adjuvant therapies seem relevant in order to enhance bony implant fixation. Ca-P coatings, HA, were introduced clinically for that specific purpose and with the intention to improve the survival rate cementless joint prostheses [215,291,380].

Calcium phosphate coatings

Experimental background

In 1977, sintered HA and other Ca-P's were proved to bond strong with bone [88,199,202]. However, due to the mechanical properties of bulk materials with low resistance to fatigue failure the material was unsuitable for load-bearing application. In 1987, de Groot et al. and others published results of plasma sprayed HA-coated bone implants [68,69,90,134,216]. It was demonstrated that HA had osteoconductive properties and that mechanical fixation of HA-coated implants were better than uncoated implants during optimal surgical conditions (press fit). Later, Søballe and coworkers demonstrated that HA coating also was capable of enhancing bone ingrowth and mechanical fixation in a gap situation during non weight-bearing and weight-bearing conditions and during stable and unstable mechanical conditions [367,370, 372-377]. Thus, the literature agrees that HA exhibits osteoconductive properties and that early bone ingrowth and mechanical fixation of bone implants

are improved by HA.

Clinical experience with HA coating is increasing but long-term follow-up remains to be evaluated. However, numerous short-term studies have shown promising results [44,121,125,132,136,215,218,236,275,291,292,340,380,390]. Thus studies using roentgen stereophotogrammetric analysis have demonstrated that HA coating is capable of reducing the early migration of both femoral hip and tibial knee components [215,218,275,291,292,380]. In addition, human retrievals have documented good bone apposition suggesting stability between implant and bone [10,12,62,371,391].

Concerns of calcium phosphate coatings

Several concerns regarding Ca-P coatings have been raised. First of all the risk of coating delamination by failure of the coating-implant interface has been suggested to cause implant loosening. Moreover, the effects of coating resorption have been advanced as a cause of reduced implant fixation. Another issue of debate is; which coating quality is optimum? Should it stay on the implant surface or should its function only be temporary. In regard to long-term or temporary performance of Ca-P coatings two research direction have been directed. One toward creating more stable coatings to enhance bonding strength between coating and implant and one toward creating more resorbable coatings to increase coating bioactivity.

Histomorphometry, stereology and bone implant research

Bone histomorphometry is one of the most important basic evaluation methods in bone implant research. However, papers often only included simple description or semi-quantitative evaluation of histology. Another problem in histomorphometry is how to get unbiased estimates and how to sample efficiently. Due to adaptation to weight-loading and stresses mature bone is anisotropic (preferred orientation) morphologically and mechanically [128,140,395,410]. When using conventional histomorphometry analysis of bone-implant surfaces, anisotropic orientation will result in biased estimates. Bias is irreversible and most often undetectable and might result in wrong conclusions [157]. However, by applying a recently developed method, *the stereological vertical section method,* unbiased estimates of anisotropic orientated surfaces are achieved [7,155,157] (I–VIII). Less attention has been payed to sampling. Histologically, sampling is necessary because a total 2D trace of 3D surface, volume or thickness are impossible. Planning of the sampling method before processing specimens and sections is important in order to have an *efficient* method and in order to get *unbiased* estimates with good precision (VIII).

Aims of studies

Effects of surface texture on implant fixation

Failure of the coating-implant interface, which might result in coating delamination and eventually production of particles contributing to the third body wear process, is both a risk and concern of HA coatings [9,21,22,43,101,119,200,201,378]. Currently, HA coatings are clinically available on prostheses with either a grit-blasted, rough or porous substrate surface. The implant surface texture might influence implant fixation and risk of coating-implant failure.

The effects of porous-coated versus grit-blasted surface texture of HA-coated implants on mechanical fixation and bone ingrowth and coating delamination were evaluated in a non weight-bearing gap model during 25 weeks (II), and in a weight-bearing model with controlled micromotion of 500 μm during 16 weeks (III).

Effects of calcium phosphate coating quality on implant fixation

Coating quality is an important factor in the biological performance of Ca-P coatings. Recently, a fluorine containing coating, FA, was shown to be more stable than HA and surprisingly FA increased bone ongrowth compared with HA [97,233,234,255]. However, FA coatings have not been investigated in clinically relevant weight-bearing models nor in humans and the effect of fluorine on bone remodeling should be elucidated. In studies on osteoporosis, fluorine has shown to have adverse effects on bone remodeling and mechanical properties of bone [26,109,256,334,355,428].

The effects of HA- versus FA-coated implants on mechanical fixation and bone ingrowth on remodeling were evaluated in a stable weight-

bearing model during 25 weeks (IV), and the effects on bone ongrowth were evaluated in humans in a non weight-bearing gap model in trabecular bone during 1 year (VI).

Most recently, it has been speculated that HA coating crystallinity influences early bone ingrowth [55,84,265]. Coating crystallinity has been shown to be an important factor for HA coating stability in vitro and in non-weight-bearing studies [76,84,233,234,265-267]. Moreover, some evidence exists that less crystalline (more resorbable) HA coatings are beneficial for early bone ingrowth. However, whether or not this will enhance implant fixation has not been investigated in weight-bearing models.

The effects of HA coating crystallinity on mechanical fixation and bone ingrowth were evaluated in a weight-bearing model with controlled micromotion of 250 μm during 16 and 32 weeks (VII).

Factors with influence on loss of calcium phosphate coatings in vivo

Despite the general belief that resorption is necessary for bone bonding to occur, it has been proposed that resorption reduces the bonding strength between implant and substrate and disintegrates the coating. This could lead to delamination and failure of implant fixation and to acceleration of the third body wear process [9,21,22,43,101,119,200,201,378]. Early studies showed no resorption of HA whereas recent reports have demonstrated that resorption does occur. However, little is known about mechanisms and factors influencing coating loss *in vivo*. The present series of studies were designed to investigate the effects of several factors on coating loss in vivo, however not to distinguish between the mechanisms of coating loss. Clinically the *mechanical factor* might play an important role in loss of coatings especially during the early postoperative period [123].

The effects of controlled continued micromotion and immobilization of the implants on coating loss were evaluated in a weight-bearing model with controlled micromotion of 150 μm during 16 weeks (I).

Effects of *coating related factors* on loss of Ca-P coating in vivo were investigated.

The effects of HA versus FA coating and of HA coating crystallinity on coating loss were evaluated (V, VI, VII).

Prosthetic related factors such as surface textures might influence loss of HA coatings.

The effect of porous-coated versus grit-blasted surface on coating loss were evaluated during both non weight-bearing and weight-bearing conditions with controlled micromotion (II, III).

Sampling efficiency and stereological estimation methods

Until recently, available methods for sectioning hard tissue with metal implants have been limited and time consuming, and only few sections could be obtained from each specimen. However, with new technology, the importance of sampling efficiency is more evident. A newly developed hard-tissue saw can produce numerous exhaustive and serially cut sections from each bone-implant specimen with a known distance between sections [232,(III,VII)]. This gives the investigator the opportunity to use more efficient and unbiased sampling strategies than today where most research is based on a single or few arbitrary sections from each implant specimen.

The efficiency of systematic sampling using the unbiased stereological vertical section method was studied. Different sampling intensities were analyzed in order to find an optimal sampling design to reduce workload, both at the hard-tissue saw, and at the microscope, on relevant sampling levels (VIII).

Biomaterials

The wide variety of biomaterials used for clinical application can be divided into 4 major categories based on chemical structure: Metals, ceramics, polymers, and composites. This review will deal with metals and ceramics. Based on the biodynamic interactions with the biological environment, Osborn and Newesely classified biomaterials into *biotolerant, bioinert, and bioactive* [88,296,413]. *A biotolerant* biomaterial is characterized by the fact that a fibrous tissue layer will always develop around the implant, so-called distance osteogenesis. *A bioinert* biomaterial has been defined as a material not having any action on the tissue. Bone will achieve contact to a bioinert material after a period of time, contact osteogenesis. Alumina, zirconia and carbon are examples of such materials. The term bioinert has been criticized because every material might elicit a response from the tissues at the interface [177]. *A bioactive* biomaterial is designed to induce a specific biological activity promoting enhanced bone formation and direct chemical bonding of bone to the material surface so-called bonding osteogenesis. Many materials have been described as exhibiting bioactive behavior such as Ca-Ps and other ceramics, polymers and titanium and its alloys might also be bioactive [162,172].

Metals

Several kinds of metals are used clinically. Most often the metallic part of the prosthetic components are made of alloys of titanium or cobalt-chrome. The biocompatibility and mechanical properties of the metals are important for early and late implant fixation [175].

Biocompatibility. The biological response to titanium and cobalt-chrome alloys have been studied extensively and their biocompatibility has been demonstrated to be favorable [45,46,207,348]. In an early study, Brånemark described the concept of osseointegration and suggested that direct contact between titanium implants and bone at LM level was achieved [30,31]. Later, Linder et al. confirmed that titanium implants could achieve osseointegration without the presence of fibrous tissue at the bone implant interface at EM level [251]. In vitro, osteoblasts grow faster on titanium-alloy (Ti-6Al-4V) than on cobalt-chrome alloy or stainless steel [322]. In addition, wear particles from Ti-6Al-4V might be less toxic than from cobalt-chrome [173]. In vivo, several studies have shown that the bonding strength between bone and implant favor titanium-alloy implants, however, no difference in bone ingrowth has been demonstrated [70,127,206]. The excellent biocompatibility of titanium might be explained by the titanium-oxide (TiO_2) layer that spontaneously forms on the implant surface [162,172]. In addition to TiO_2 which might vary from a few nm to 200 nm in thickness, Ca and P will bind to the surface creating a very thin layer of apatite.

Mechanical properties. The elastic modulus (stiffness) of the prosthetic components might influence the stress distribution in the bone which will modulate bone remodeling. The elastic modulus (stiffness) of cobalt-chrome-molybden (CoCr-Mo) (210,000 MPa) is almost twice as high as Ti-6Al-4V (110,000 MPa) which is 5 times higher than that of cortical bone (20,000 MPA). By use of finite element modeling bone resorption was found to be much greater for CoCrMo than for Ti-6Al-4V stems due to stress shielding [175,407]. In contrast, very flexible stems with a stiffness close to bone might increase micromovements at the interface which might interfere with bone ingrowth.

Corrosion. It is well established that all metals are subjected to corrosion and that porous coatings exhibit higher corrosion rates compared with non-porous metals. Commercially pure titanium and its alloys, most often Ti-6Al-4V, are the most corrosion-resistant [130,207,414]. Under passive conditions corrosion of titanium alloy is 2–3 times lower than that of cobalt-chrome alloy [130].

Tribological properties. Poor tribological properties might result in increased production of wear particles. The use of titanium as the bearing sur-

face in total joint replacements has been minimized after reports of aseptic loosenings associated with excessive wear of titanium femoral heads whereas CoCrMo heads are widely used [418]. It is beyond the scope of the current review to present a detailed discussion on wear and effects of wear particles, but several papers have been published on this issue [2,70,142,163,173,188,195,261-263,270,345].

Conclusion. Currently, it seems that corrosion, biocompatibility and mechanical properties of metals favor titanium alloy rather than cobalt-chrome [175]. New titanium-based alloys are under investigation to eliminate aluminum and vanadium to enhance the fatigue properties and to reduce corrosion and cytotoxicity [194]. Tantalum might be used alone due to better tribological properties than titanium [207]. Recently, implants with porous coatings of tantalum have demonstrated excellent bone ingrowth [25,32,194,208,349,358].

Metal surface textures

The surface texture can be divided into 3 types based on surface preparation method [24,70,313]: Polished, grit-blasted, and porous. A number of implant designs with irregular macro-surface texture but non-porous surfaces exist. Only grit-blasted and porous-coated surfaces will be dealt with in this review as relevant to Ca-P coatings and cementless implant fixation, since coatings on smooths surfaces have failed.

Porous coatings. Porous coatings were developed to create a possible method for fixation of prosthetic components by bone ingrowth into the void spaces and channels of a porous material and to reduce stresses on the implant surface [127]. The porous coatings are characterized by a three-dimensional interconnecting pore system which allows bone ingrowth [24,70,127,312,313,360]. A kind of pore system has also been demonstrated between cement and bone due to interdigitation of cement with the texture of trabecular bone but not with cortical bone as reported by Charnley [52,53]. The porous *microstructure* is an important parameter for bone ingrowth. Klawitter et al. demonstrated that a porous implant required a minimum interconnecting *pore size* of 100 μm to obtain bone ingrowth [224]. It is generally accepted that the optimum *pore size* range is 100 to 500 μm [24]. *The porosity* of a plasma sprayed porous coating is dense near the substrate and more open at the outer surface. No matter which metal is used a porous structure will be weaker than the solid [65,161,313]. Clinically, failure of bead porous coatings of cobalt-chrome have been reported [54,161]. The *thickness* of the porous coating might be important for implant fixation. Cook et al. found increased shear strength as the number of powder particle layers increased from 1 to 2 and from 2 to 3 [70].

Three types of porous coatings exist: Plasma-sprayed, sintered bead coatings, and diffusion bonded wire mesh (fiber-metal) porous coating, [160,313,317,360]. In addition, a new type of porous coating made of tantalum has been introduced [25,32,194,208,349,358]. It is manufactured by deposition of tantalum on a carbon matrix resulting in a very porous metal coating. *Plasma sprayed porous coating* consists of a continuous dense part which interlocks with the surface but without metallurgical bonding. Several parameters can be varied during processing resulting in coatings with different microstructures. The plasma sprayed coating has a very irregular surface compared with the sintered and diffusion bonded porous coating. *Sintered bead porous coating* is manufactured at very high temperatures resulting in metallurgical bonding of the beads to the substrate surface. Spherical particles from 50–1000 μm of titanium and cobalt-chrome alloy are used. *Diffusion bonding (fiber-metal) porous coating* consists of molded metal fibers which are sintered onto the metal surface in combination with pressure to obtain metallurgic bonds at contact points [127,129]. The fiber-metal coating differ from the bead coating in that the porous surface is more compliant which might result in lower stresses at the bone implant interface [313].

Recently, Noble et al. compared different types of porous coatings and found that a plasma-sprayed titanium coating significantly reduced interface micromotion of acetabular cups compared to a Co-Cr bead coating [280]. In addition, fatigue strength of plasma-sprayed porous coatings has been shown to be better than other coatings.

Grit-blasted surfaces. Grit-blasting is used in combination with various coating methods for preparation of the implant surface. Moreover, parts of the prosthetic components for cementless or cemented use can be grit-blasted. Very few

Table 1. Classification of ceramics categorized into bioinert and bioactive types.

Mineral	Name of compound	Abbreviation	Formula	Ca/P ratio
Bioinert oxide ceramics				
Alumina	Aluminum oxide		Al_20_3	
Zirconia	Zirconia oxide		ZrO_2	
Bioactive ceramics				
Glasses				
Bioglass	Silicium oxide		SiO_2 CaO Na_2O P_2O_5	
A/W glass ceramic	Oxyapatite and wollastonite		MgO CaO SiO_2 P_2O_5 CaF_2	
Calcium phosphates				
Whitelockite	Tricalcium phosphate	TCP	$Ca_3(PO_4)_2$	1.5
Hydroxyapatite	Pentacalcium-hydroxy-triphosphate	HA	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Fluorapatite	Pentacalcium-fluoride-triphosphate	FA	$Ca_{10}(PO_4)_6F_2$	1.67
Hilgenstockite	Tetracalcium phosphate	TTCP	$CaO.Ca_3(PO_4)_2$	2.0

prostheses for cementless application are solely grit-blasted [20]. Grit-blasting is essential for bone ongrowth and implants fixation as compared to a polished implant surface [117]. Currently, several different grit sizes are used (0.1–0.5 mm in diameter) resulting in different surface textures [412].

Ceramics

Ceramics are a broad variety of materials of non-metallic and inorganic origin which have been used clinically for several years. Ceramics can be categorized into bioinert and bioactive types (Table 1). *Bioinert oxide ceramics* like aluminum oxide (Al_20_3) and zirconia oxide (ZrO_2) are used as bearing surfaces in joint replacements due to very good wear rate properties [417]. The material has high resistance to corrosion, is not deformable, and has good fatigue properties. A disadvantage is that the material is brittle. *Bioactive ceramics* are constituted of several different Ca-P's and glass types including monophase glasses such as Bioglass and glasses. The glasses might have several phases like glass-ceramics (A/W glass ceramic) [223,426]. These ceramics are available as bulk materials and as coatings and have been investigated extensively [178,222,426].

Calcium phosphate ceramics. Ca-P's are the most important bioactive ceramics for this thesis and several types of Ca-P's have been investigated (Table 1). Chemically, the constitution of HA, TCP and FA is close to that found in bone [242,318,319]. Ca-P's can be classified into four groups: Granula or particles, solid or bulk forms, composites, and Ca-P coatings. Clinically, Ca-P materials are most often used as a bone filler in non weight-bearing situations, except when it is applied as a coating [36,185]. *Granula or particles* are made of HA or TCP and have been used especially in dental surgery for mandibular and maxillary ridge augmentation [315]. *Solid or bulk* HA is manufactured by compression of HA powder followed by heat-treatment [89]. Pores are produced by mixing the starting powder with H_2O_2. Bulk materials have high compressive and tensile strengths (up to 600–700 MPa and 200–250 MPa, respectively) compared to bone. However, the resistance to fatigue failure is very low because of low deformation capacity [90]. Coral is a natural existing bulk Ca-P with interconnecting pores. Hydrothermal exchange methods convert coral into HA [184]. Coral has been used clinically for several years [184]. *Composite materials* of Ca-P and other materials exist. HA incorporated in polylactic acid has been used as bone filler and drug carrier [91,180].

Calcium phosphate coatings

Several synthetic Ca-P coatings exist; among these HA, FA and TCP coatings have been investigated most extensively (Table 1). FA exhibits the same apatite structure as HA, but the hydroxyl groups have been replaced by fluorine ions resulting in a more compact lattice (Figure 1). The plasma spraying process of FA coating has been reported to be more stable at high temperatures compared to HA, which makes FA suitable for the coating procedure [255]. TCP is chemically almost

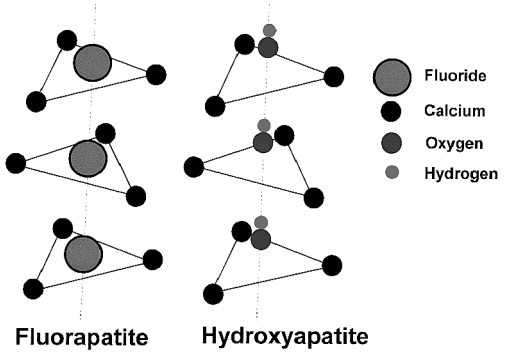


Figure 1. Partial crystal lattices of hydroxyapatite (HA) and fluorapatite (FA) arranged around the vertical axis of the crystals [319]. The calcium ion lattice of FA is identical to HA except for the position of F and OH. The F ions are surrounded by calcium ions resulting in a more compact lattice of FA than HA and stronger electrostatic bonds between calcium and F ions than between calcium and OH ions.

similar to HA and has the crystal structure of ß-whitelockite.

Application methods

Several different techniques, resulting in different coating qualitites are used for application of Ca-P coatings [15,16,179,193,210,241,384,398,408]. Each of the techniques has its own technical limitations, and so far, an optimal method for producing a bioactive coating with high bonding strength on a porous implant remains to be developed. This review does not intend to present a comprehensive list of methods but the most significant currently employed will be described briefly.

Plasma sprayed coatings. The plasma spraying technique has been used in various fields since 1970 [179]. Plasma-sprayed HA coatings were introduced in the 1980's and currently comprise the most frequently used method for clinical application of Ca-P's coatings [16,89,384] (Figure 2). Before plasma-spraying, the implant surface is roughened by grit-blasting, shot peening, or by etching. The starting powder is important for the coating quality and should be pure with high crystallinity [388]. The plasma spraying process is very critical for the coating quality and several parameters have to be controlled [34,152,179]. The final coating crystallinity depends on the particle temperature in the plasma flame, on the particle position within the flame, the flame velocity, residence time in the flame and on the particle size. Crystallinity changes also with the distance between spray gun and substrate. Overheated particles will cause evaporation or chemical deposition of the coating material and will result in impurities and a less crystalline coating. Insufficient heating causes a coating with poor bonding to the substrate surface and with high porosity and impurities of TCP. The stability of the meltened powder in the plasma is determined by its chemical composition. FA powder is more stable than HA at critical temperatures whereas HA dissociates more easily into tricalcium phosphate resulting in more impurities [255].

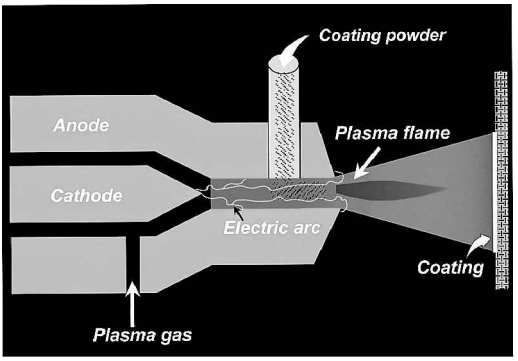


Figure 2. **Schematic drawing of the plasma-spraying process**. The plasma gun consists of a cathode and an anode [179]. An electric arc is created between the electrodes while a stream of gas (usually argon) passes through. The gas is ionized by the electric arc and is transformed into a plasma (a collection of rapidly moving ions and energetic electrons). The temperature of the plasma is extremely high, 20–30,000 °C, and the speed is approximately 300 m/s. Coating powder is fed into the plasma flame. The powder melts when it reaches the plasma and is then conveyed to the substrate. On the substrate surface the particles form splats (solitary droplets) which melt together and bond to the surface as a coating. The substrate is kept "cold", usually less than 300 °C for metals, to preserve the characteristics of the implant.

Post-heat treatment of plasma-sprayed HA coatings results in more crystalline coatings and reduction of TCP impurities, whereas FA coatings hardly change [152,205,234]. Heat-treatment has shown to improve bonding strength by further diffusion of the coating into the substrate surface [205,244]. Other plasma spraying methods such as vacuum plasma spraying and high velocity flame sprayed coatings have been developed during recent years [286,287,347]. These methods produce coatings with good adhesion strength and few pores, however they might overheat the metal substrate, thus changing the mechanical properties.

A disadvantage of the plasma spraying process is that only surfaces exposed to the plasma flame are covered with the coating. Hence, a porous-coated implant surface coated with HA has surfaces without HA. Another disadvantage is that the thickness of the coatings should be at least 40–50 μm in order to have a homogeneous surface coverage. Thinner coatings would be uneven due to the particle size of the starting powder.

Sputter coatings. To overcome some disadvantages with the plasma spraying technique of HA coatings, sputter coatings (magnetron or ion beam) have been developed [238,398,422]. The coatings have excellent adhesion strength. A uniform coating is achieved by the method irrespective surface topology and the thickness of the coating might be very thin (2–10 μm). A disadvantage might be that a sputter coating at the substrate interface consists of an amorphous phase of a few nanometers; moreover, the production time is long.

Solution precipitated and other coating methods. Several different methods for solution precipitated coatings have been published [1,238,408]. Generally, the coatings are produced by immersing the implant into different aqueous supersaturated calcification solutions to mimic natural apatite formation. Some of the methods are conducted at low temperatures and result in a thin coating which covers the total implant surface. While crystallinity is high, the bonding strength might be low. *Dip-coating* is processed dipping the implant into a slurry of HA followed by a sintering process [238]. A variant of the sintering method is hot isostatic pressing at a maximum temperature of 850 °C. The coatings are applied under high pressure which has been shown to increase bonding strength [282,411].

Quality assessment of calcium phosphate coatings

Significant variation occurs in the quality of HA coatings from different companies and batches although that coating quality has been shown to influence bone ingrowth, mechanical fixation and resorption of the coating [76,244]. In addition, several factors are critical to the physicochemical behavior of plasma-sprayed coatings. Thus the coating quality should be characterized appropriately (Table 2). Currently, several proposed standards (ISO, FDA, ASTM) on HA coatings have been published, but few have been approved [5,99,116]. XRD analysis, scanning electron microscopy and energy dispersive spectrometry, and Fourier transform infrared spectrometry are all used for quality assessment of the Ca-P coatings.

Table 2. Standards for hydroxyapatite (HA) coating quality according to [a] ASTM (The American Society for Testing and Materials) and guidelines proposed by [b] FDA and [c] ISO (International Organization for Standardization) in draft forms [5,99,116].

Parameter	Standard for HA coating
Chemical composition	$Ca_{10}(PO_4)_6(OH)_2$ [a,b,c]
Purity	>95% HA [a,b,c]
Trace element content	<50 ppm of heavy metals [a,b,c]
Ca/P ratio	1.67–1.76 [a], 1.67 [c]
Crystallinity	>62% [b] and >45% [c]
Solubility	Test at pH of 3.0 and 7.3 [b]
Density	2.98 g/cm^3 [b]
Porosity	5–20% [d]
Thickness of coating	50–100 μm [d]
Mechanical properties	
Shear strength	>22 MPa [b], >15 MPa [c]
Tensile strength	> 51 MPa [b]

[d] No standard available, figures proposed from the existing literature on plasma-sprayed coatings.

Chemical composition, purity, trace elements and Ca/P ratio. The chemical composition of HA must be equivalent to the stoichiometric formula [$Ca_{10}(PO_4)_6(OH)_2$] according to the ASTM standards [5]. Purity has to reach a minimum of 95% HA, the remaining part being tricalcium phosphate, tetracalcium phosphate, calcium oxide, or calcium pyrophosphate. Trace element analysis must be limited to 50 ppm of heavy metals according to the draft FDA protocol [116]. The Ca/P ratios in atomic percent should be 10/6 = 1.66–1.67 for the powder (stoichiometric HA) and 1.67–1.76 for the coating.

Crystallinity. XRD analysis is the state of the art for evaluation of coating crystallinity (Figure 3) [321]. Most often assessment is done on coupons and not on the coated implant. Briefly described, an X-ray beam with a known wavelength is passed through a sample of the coating and diffracted beams are recorded by a detector. The lattices, specific for every crystals, produce a unique pattern of peaks or reflections of a certain intensity at

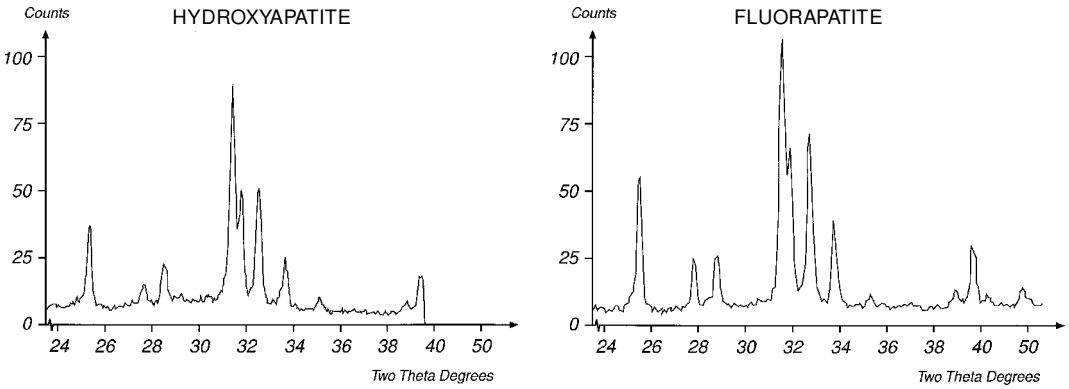


Figure 3. XRD patterns of hydroxyapatite and fluorapatite coatings from Study II–VI. Note, line broadening of the curves indicating that parts of the coatings are amorphous phases.

specific angles. Crystallinity can be calculated from the X-ray diffraction pattern by dividing the integrating area under the curve between wave lengths (2Θ of 30% and 2Θ of 35%) with the results obtained from a control specimen. Coatings with high crystallinity have sharp narrow peaks with high intensities, whereas more amorphous coatings have lower intensity and broader peaks with shoulders [244]. XRD analysis can detect other crystalline phases like TCP, TTCP and CaP when present above 1 weight %.

Ca-Ps exist in phases from completely amorphous coatings to coatings with almost 100% crystallinity. The degree of crystallinity is defined as the ratio between crystalline and non-crystalline (amorphous phase) coherent scattering acquired from XRD analysis as described above. By this method the mean crystallinity of the entire coating thickness is estimated but heterogenetics in coating crystallinity is not revealed [310]. Often the most profound part close to the substrate is less crystalline than the superficial part due to changes during the plasma spraying process [150,152]. Currently, the FDA guidelines describe how to assess crystallinity by XRD analyzes and recommend that coating crystallinity should be 62% as a minimum. According to the manufacturer, the crystallinity of plasma-sprayed coatings available on the market varies between 60 and 80%, whereas solution precipitated coatings have higher crystallinity [76]. Company data must be interpreted with caution because crystallinity assessment might be done by different methods which might result in different figures for identical coatings [244].

HA crystallinity might be increased from amorphous to a highly crystalline coating during 12 weeks incubation in Ringers solution as demonstrated by Gross and Berndt [149]. Biologically, HA coating crystallinity might play a role in early bioactivity, and crystallinity is important for the coating solubility [82,84,265] (VII).

Solubility. Small changes in chemical composition, porosity and crystallinity of the coating can influence dissolution characteristics considerably [100,225,227,230,233,266,310]. Solubility testing should be conducted in buffer solvents at a pH of 3.0 and 7.3 [116]. Dissolution kinetics are influenced by the coating quality and type in addition to the microenvironment. Coating solubility will increase when impurities are present. Moreover, a low pH accelerates dissolution of Ca-P coatings, especially those with low crystallinity. Other factors like crystal structure, neck geometry, and grain size will also influence solubility [225,230]. FA coatings have shown less solubility than HA particularly at low pH [100]. By contrast, TCP crystals are highly soluble and unstable at physiological pH. Recently, Paschalis et al. demonstrated that dissolution rates of HA coating from 6 different vendors differed by a factor of 5 although that XRD indicated similar crystallinity before and after solution treatment [310].

Porosity. Porosity is defined as the volume fraction of the coating without Ca-P and is assessed by scanning electron microscopy in backscatter mode of a cross sectioned coating. Porosity corresponds to density which is defined as coating weight per volume (g/cm^3). Porosity is established during processing when powder particles

are not completely melted and is related to the particle size of the powder. Coating porosity is of importance for the mechanical properties and for resorption of Ca-P coatings [229]. Thus, tensile and compression strength depend exponentially on porosity [89]: the higher the porosity the lower the strength. Because Ca-P coatings are brittle, pores do not weaken the material in terms of crack propagation but will disrupt the crack extension when the implant is strained [179].

Coating thickness. Optimum coating thickness depends on the coating quality, function and design of the implant. From a mechanical point of view, it is preferable to have a thin coating since it reduces the risk of coating fracture and it preserves the porous surface structure of an implant. Coatings in excess of 100 μm behave like a brittle ceramic and are at particular risk of mechanical failure [347]. In vivo, experimental data have shown that a thin coating of 50 μm resulted in higher shear strength than a thick coating of 200 μm [367,404]. Failure mode changes with coating thickness. Wang et al. showed that a 50 μm coating failed at the bone-HA interface whereas a 200 μm thick coating failed at the implant-HA interface and within the coating. Fatigue test of hip stems with 120 and 240 μm HA coatings showed severe delamination and cracking at loads of 30% of the stem yield strength, whereas no damage was demonstrated on the 50 μm coating [217,347]. No consensus exists as to how thick a coating should be but most coatings on the market are probably 50–100 μm thick.

Significance of calcium phosphate coating on corrosion and ion release. Reduction of ion release from metals during implantation is favorable. It has been demonstrated that plasma-sprayed HA coating significantly reduced the ion rate release from porous-coated titanium alloy whereas no major changes in ion release from Co-Cr alloys occur [103,330]. This beneficial effect can be explained by a shielding effect, however, altered kinetics of metal ion release seems more likely due to changes in surface oxidation, especially on the titanium surface.

Mechanical properties. The bonding strength between coating and metal substrate is very important for weight-bearing implants [34,165,210]. The precise mechanisms of attachment forces between Ca-P coating and metal is under investigation [210]. It has been demonstrated that HA reacts with titanium oxide at elevated temperatures (800–1000 °C) creating a chemical bonding, whereas the reaction with Co-Cr alloy is less significant [89,103,118,193]. This might explain the fact that HA coating on titanium demonstrated higher bonding strength and better fatigue properties than on cobalt-chrome [197,237]. Tensile and shear strength of the coating are reduced significantly by increasing thickness [217,347,404,423].

Bonding strength is tested using coupons with a grit-blasted surface by gluing the HA-coated substrate with various epoxy or methacrylate glues [89,118,357,423]. Coating strength varies with adhesive type probably due to different penetration depth of the glues into the coating and is inversely related to coating porosity and coating thickness. Moreover, the underlying substrate surface roughness seems to be important [118]. It is assumed that the bonding strength is higher on a porous surface supported by the observation that tensile strength of an HA coating is lower on a polished implant surface compared with a grit-blasted [423]. However, testing of a porous-coated surface is not possible because it would be a test of the porous coating and the glue instead of the Ca-P coating. With regard to standards for fatigue and abrasion testing, these are only available in draft forms although the failure modes are essential for long-term durability of weight-bearing prostheses [89, 99,116]. It is proposed that testing be done on the worst case scenario and that femoral stems be tested at a load of 3–4 times body weight for 10 million cycles. Fatigue testing of HA and FA coatings (RR Moore rotating beam test) showed that dry test conditions lead to no coating failure whereas wet conditions (physiological solutions) lead to large coating delamination, which emphasizes the significance of test conditions [59,174,237]. According to the standards, shear strength must be more than 15–22 MPa and tensile strength should have a minimum of 51 MPa irrespective type of surface texture. Key parameters for the mechanical properties and fixation of the coating are: Coating thickness, porosity, surface texture and the design of the prosthetic component [89,217,347,423]. Factors which influence coating resorption may also affect bonding strength, ie. rapid resorption results in decreased bonding strength [427].

Bone implant interface biology

Bone structure and function

Bone is a highly specialized connective tissue that serves mainly three functions: 1) *mechanical*, support and site for muscle attachment for locomotion; 2) *protection* of vital organs and bone marrow; 3) *metabolic,* reserve of ions especially Ca-P and magnesium which is essential for several cell functions. Bone consists of cells and extracellular matrix of which 60–70% is non-organic material whereas 30–40% is organic [318,319,327,332]. The non-organic part is mainly HA which consists of different crystalline phases with Ca/P ratios from 1.5 to 1.7. In addition, there is a phase of non-crystalline amorphous HA. In bone, carbonate [CO_3^{2-}] substitutes for the hydroxyl groups [OH^-] reducing the crystal size which increases solubility of bone apatite especially at low pH. Simultaneously with HA, several other Ca-P's, such as TCP, are present in bone apatite [87,242]. While bone apatite is comparable to that of HA coatings, however, several characteristics are different. Firstly, HA in bone is more inhomogeneous with smaller crystal size and lack of crystal and chemical perfection. Moreover, trabecular bone has a large surface area varying from 100 to 200 m^2/g which makes bone mineral more reactive than HA coatings.

The organic part of bone consists mainly of collagen (90%) of which type I is the dominating. Several other non-collagen proteins are synthesized by bone cells or are exogenously derived and entrapped in the bone matrix. Proteins like osteonectin, osteocalcin, fibronectin, thrombospondin, bone sialoprotein and protoglycans are important for the binding of calcium ions and affect cell attachment and spreading [108,148,252,328]. Growth factors are another group of proteins which regulate bone remodeling and repair (see later).

Cortical and trabecular bone are mature bone arranged in parallel lamellar constituted by collagen fibers which are apparent when viewed by polarization microscopy. [35,48,409,410]. Woven bone is immature and is characterized by more randomly oriented collagen fibers which subsequently turn into lamellar bone after remodeling. Woven bone is more flexible, more easily deformed, and weaker than lamellar bone [420]. Biologically, trabecular bone accounts for 50% of the total bone metabolism despite the fact that trabecular bone comprises only 20% of the total bone mass [112]. Biomechanically, bone function is provided by orientation of bone trabeculae and osteons which makes bone anisotropic, i.e. with a preferred orientation, both mechanically and morphologically, [35,128,410,416].

Bone healing/repair around implants

The interactions in the bone implant interface are initiated from the time of implant insertion. The complex physiologic processes, comparable to those of fracture healing, are regulated by numerous different factors and involves participation of several cell types (Figure 4) [80]. The biological response can—according to fracture healing—be divided into primary and secondary healing [108]. *Primary healing* involves a direct healing without formation of callus. Primary healing seems to occur only when optimum conditions exist, i.e. mechanical stability and no presence of gaps; in fracture healing anatomical restoration of the bone fragments is needed. When such conditions are present, the remodeling unit (cutting cones) with osteoclasts will reestablish the haversian canals between the bone ends while the osteoblasts form bone [268]. *Secondary fracture healing* which is supposed to take place around cementless implants occurs when optimum conditions for repair are absent and involves the formation of callus. Histologically, several phases in the process of secondary fracture healing and at the bone-implant interface have been described [4,37,38,94,108]. Initially, a hematoma is formed and the inflammatoric response commences (Figure 4). The hematoma is suggested to be a source of signaling molecules which are released from platelets and

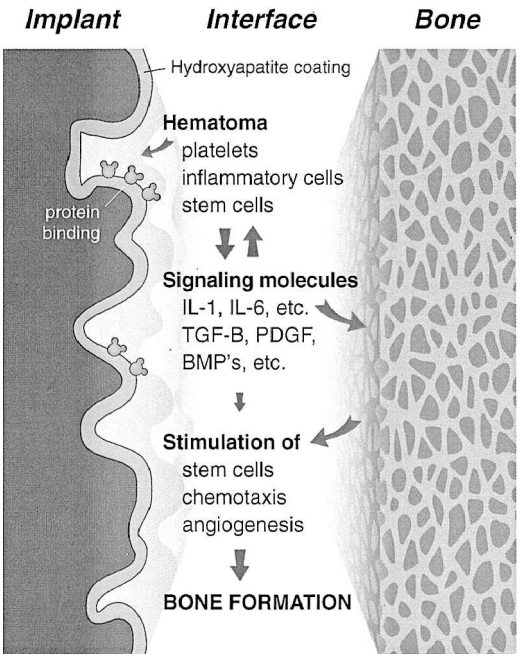


Figure 4. **Schematic drawing of the bone implant interface.** The processes and interactions at the bone implant interface are initiated from the time the implant is inserted. Initially, a hematoma is formed. Signaling molecules are released from inflammatoric cells and platelets, regulating and stimulating mesenchymal cells, chemotaxis and the angiogenesis. The most important molecules are most likely cytokines such as IL-1 and 6 in addition to several growth factors like transforming growth factor-beta (TGF-ß), platelet derived growth factor (PDGF), insulin-like growth factor (IGF) and bone morphogenetic proteins (BMP's). Extracellular matrix proteins are also important for the repair processes and will bind to the implant surface. They might stimulate chemotaxis and enhance cell binding to the implant surface. Bone formation is the net result of these interactions.

inflammatoric cells. The signaling molecules are important for the early cellular regulation of the repair process by stimulating mesenchymal cells and the angiogenesis whereas expression of e.g. bone morphogenetic proteins (BMP's) in osteoblast decrease later when woven bone is converted to lamellar bone [108]. The most important molecules are most likely cytokines such as IL-1 and 6 in addition to several growth factors like transforming growth factor-beta (TGF-ß), platelet derived growth factor (PDGF), insulin-like growth factor (IGF) and BMP's. Extracellular matrix proteins might also be important for bone implant interface biology [108,148,252,328]. It has been suggested that fibronectin plays an important role in governing the interactions of the implant surface and the surrounding matrix [257]. The hematoma will be invaded by cells and vessels, and callus formation begins after 7–14 days [94,346]. Osteoclastic resorption of the interface bone has been observed as one of the most dominating processes during the first week after insertion of an implant in cortical bone. This might explain why BMP-7 decreased the mechanical fixation of primary inserted implants compared with implants not treated with BMP-7 [368]. The formation of callus is dependent on mechanical conditions and distance, presence of gap, between implant and bone.

Mechanical stabilization and gap healing. During *stable mechanical conditions* without gaps, intramembraneous bone formation will take place directly after the inflammatoric response [30,94]. The presence of a gap over a certain size creates a different situation. It seems that small defects less than 0.5 mm in diameter heals by direct intramembraneous bone formation, whereas larger gaps will heal through the cartilage stage and an initial scaffold of woven bone which subsequently turn into lamellar bone. In both situations, the newly formed bone adapts to the new situation by orienting of the bone architecture. During *unstable mechanical conditions* the inflammatoric response is prolonged and a fibrous tissue membrane might develop [370,375] (I, III, VII). The magnitude of continuous micromotion in combination with the local environment will determine whether the inflammatoric response turns into formation of chondrocytes and endochondral ossification (secondary fracture healing) [4,42]. Preferred orientation of collagen fibrils radiating from the implant surface seems to be a predictor for later subsequent endochondral ossification which may occur from 8 to 16 weeks after the initial operation in the presence of micromovements of 150–500 μm [4,307,308,370]. The bone repair process around an implant varies considerably between humans and animals and between different models. During stable conditions bone ingrowth is achieved at 4 weeks whereas an unstable situation will delay the process and might even lead to non-union and fibrous anchorage of an implant [4,370,372] (III). Complete restoration of bone architecture in dogs might last for more than 6 months under stable weight-bearing conditions (IV).

Other factors than mechanical stability and the presence of gaps might influence bone repair; such factors include access of joint fluid to the periimplant gap, weight-bearing conditions resulting in various stress at the bone implant interface (loading versus unloaded implants), implantation site (cortical versus trabecular bone) and status of host bone. Application of substances able to enhance repair might also influence bone repair [47,367].

Local enhancement of bone repair

Bone repair can be enhanced by three basically different methods which are of interest for bone implant research: Osteogenesis, osteoinduction, and osteoconduction [33,107]. *Osteogenic* factors stimulate local bone formation. Among osteogenic factors are autologous or allogenic bone marrow and bone graft, freeze-dried allograft, and demineralized bone matrix. Furthermore, Ca-P coatings are hypothesized to have osteogenic characteristics [382]. Osteogenic factors have been applied with success in the bone implant interface [219,377,393,419]. *Osteoinduction* is characterized by stimulation of mitogenesis of undifferentiated perivascular cells leading to the formation of osteoprogenitor cells and subsequently osteoblasts. Osteoinductive factors are able to stimulate bone formation extraskeletally by recruitment of mesenchymal cells without the presence of osteogenic or osteoconductive substances. Bone autograft, bone precursor cells and bone derived growth factors are known to be osteoinductive. Among bone derived growth factors, the TGF-ß (transforming growth factor- beta) superfamily (TGF-ß and bone morphogenetic proteins (BMP's)) have selective properties to stimulate stem cells to differentiate into osteoblasts. Experimentally, by exogenous local delivery systems, BMP-7 (Osteogenic Protein 1 (OP-1)) and BMP-2 in addition to transforming growth factor-beta (TGF-ß), have been shown to enhance bone ingrowth and mechanical fixation of implants [66,246,247,249,362]. *Osteoconduction* is a process where the osteoconductive material serves as a passive scaffold which enhances and supports bone formation. This process is characterized by an initial invasion of fibro-vascular tissue into a porous structure or along a surface which subsequently is followed by new bone formation. The factors that initiate the process are poorly understood. Auto- or allograft are basic osteoconductive materials in addition to Ca-P materials such as coatings and bulk porous substrates [219,404]. Moreover, collagen, polymers, bioglasses and several metals elicit osteoconductive properties.

Combinations of materials with osteogenic, inductive and osteoconductive properties are considered in the treatment of bone defects, gaps around implants and in spine fusions. Experimental studies have shown promising results [204].

Biological response to calcium phosphate coatings

Theory of the osteoconductive properties of calcium phosphate coatings

Substantial documentation on the osteoconductive properties of Ca-P coatings exists. However, the mechanisms behind are not fully understood, but research during recent years have provided evidence of several processes [74,78,101,102,243] (Figure 5). It has been shown, repetitively, that a carbonated apatite layer is generated at the surface. It is believed that formation of this layer involve a local degradation of the Ca-P surface followed by secondary crystal growth between the artificial HA and bone and moreover reprecipitation might occur leading to a physico-chemical bonding mechanism [73,243]. Histological findings suggests that a cellular mechanism is involved in these processes probably through chemotaxis. Recently, it has been hypothesized that bone formation on Ca-P coatings is due to selective adsorption of serum protein to the surface which might be responsible for enhance osteoblast adhesion [110]. At the ultrastructual level, a cascade of early events has been suggested at the implant surface [80,396]. The early formation of an afibrillar globular calcified layer on the implant surface produced by osteoblasts seems to be crucial for bonding of bone to the implant surface. This layer fuse to form a homogenous line analogous with cement lines or lamina limitans with which collagen fibres become incorporated. Formation of carbonate-containing apatite crystals has been observed both in bone and extraskeletally, whereas as true bone formation as

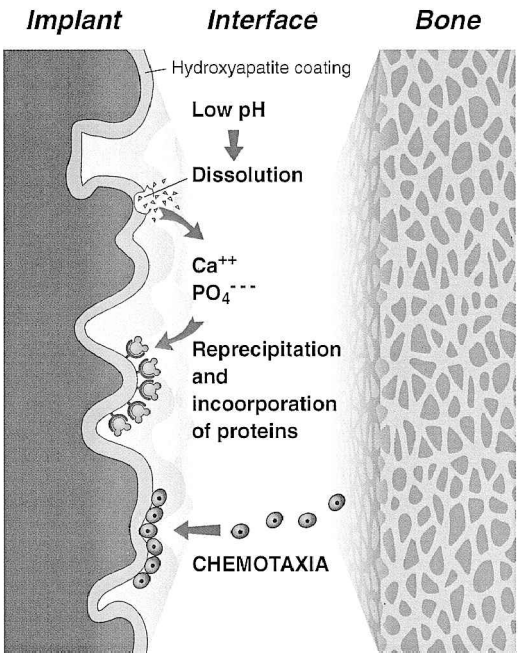


Figure 5. **Theory of the osteoconductive properties of calcium phosphate (Ca-P) coatings.** It is believed that the first event at the interface between a Ca-P coating and host bone is caused by decrease in pH resulting in partial dissolution of the coating surface. The calcium and phosphate ion concentration increases leading to ion-exchange with the microenvironment triggering reprecipitation of crystals. Ions from the extracellular fluid are incorporated into the apatite crystals together with the collagenous matrix of the new formed bone [243]. Increased concentrations of Ca and P ions stimulate chemotaxis. Moreover it has been suggested that protein adsorption might enhance osteoblast adhesion to the Ca-P coated implant surface [110].

characterized histologically has only been demonstrated in bony sites [243]. This confirms that Ca-P coatings only have osteoconductive properties.

Surface bioactivity has been investigated intensively in simulated body fluids and other buffer solvents. The induction time from in vitro incubation to formation of apatite crystals on a Ca-P coating depends on several factors such as stoichiometric deviations, crystallinity, and carbonate content [102,177,192]. The apatite layer on a non-crystalline HA coating is formed immediately, while apatite formation on a more crystalline coating is delectable after hours of incubation in supersaturated solutions relevant to the biological situation, which indicates that less crystalline might be more bioactive than high crystalline coatings [323].

Biological performance of hydroxyapatite- and fluorapatite-coated implants

Numerous investigations on HA and FA have demonstrated no adverse reactions neither in clinical nor in experimental canine and human studies when placed in bone [14,81,86,97,105,135,166,176,231,266,322,342,371,396]. Plasma-sprayed HA and FA coatings on metal implants have been shown to enhance implant fixation and bony ingrowth compared to uncoated implants in various situations [69,77,97,104,134,135,233,370,372,375,381]. Stable, press fit inserted implants achieve bony fixation regardless of whether or not they are coated with HA and interface kinetics studies have shown no difference in bone ingrowth rates [94]. By contrast, in the presence of large gaps and during unstable mechanical conditions, it can be questioned whether a metal implant will reach mechanical stability whereas HA-coated implants have been shown to be bony fixed between 8 and 16 weeks after implantation [372] (III, VI). It seems that micromovements of 500 μm in the axial direction in the presence of a 0.75 mm gap approaches the capability of HA-coated implants to become fixed by bone ingrowth whereas micromovements of 150 μm might inhibit bone ingrowth to uncoated Ti implants (III). [372,375]. In non weight-bearing press fit models, the time-dependent effect of HA and FA levels off 6–12 weeks after implantation, whereas uncoated titanium implants reach same maximal strength several weeks later [96,212,367,404].

Mainly two interfaces contribute to mechanical implant fixation: the *implant-coating interface*, and the *coating-bone interface*. Regarding the *implant-coating interface*, the mechanical properties of the coating and the bonding strength to the metal substrate are important. The bonding strength of the implant-coating interface is reduced in vivo, most likely due to disintegration of the coating because of resorption of part of the coating [212,404]. Moreover recrystallization of the amorphous phase in Ca-P coatings might result in stress accumulation leading to reduced strength [284,285]. Rapidly resorbable coatings such as TCP have shown to exhibit poor fixation compared with HA-coated implants although the implants had equal bone ingrowth [122,228,231,245]. Surface texture does influence the bonding strength of the coating to the implant surface (II, III) [118,293,357].

We showed that the HA coating delaminated on a grit-blasted surface whereas the coating stayed on the porous-coated surface. The *coating- bone interface* is supposed to be bonded physico-chemically as described above. Analyses of implants subjected to push-out test have shown bone fragments on the implant surface demonstrating that bone itself might be weaker than the coating-bone interface [96] (II, III).

Loss of calcium phosphate coating in vivo. Until recently, few studies have dealt with resorption of the HA coatings. Klein et al. investigated resorption of bulk Ca-Ps systematically [229]. The early studies investigating resorption of HA claimed that no resorption occurred in vivo [69,171,228]. However, resorption was later found in several studies and it is now well-documented that resorption of HA coatings takes place in vivo [76,97,198,231,265,267] (I, II, III, V, VI, VII). The mechanisms of coating loss can be divided into three subgroups according to the in vivo process: *Simple dissolution, cell-mediated resorption, and mechanical removal.* Several factors might contribute to coating loss. Both issues will be dealt with in the discussion part.

Methodological considerations

Experimental subjects

Animals. In the research of bone-implant fixation, several different animal species are used. Phylogenetically, low animals like rats and rabbits are advantageous because homogenous populations are available at low costs. However, for more clinically related studies, a larger and phylogenetically higher ranked animal is needed. The dog was chosen as the experimental animal because canine bone structure and vascularity are comparable to that of humans. Moreover, the employed models required a certain bone size in order to have sufficient samples for mechanical testing and histomorphometry. Finally, we have at our institution a substantial number of reports on basic canine bone patophysiology, vascularity at our disposal as well as reports on bone-implant research in dogs [41,245-247,249,324,367,369,370,372-377,379,380].

In a clinical context, the animal model has several advantages. The animals are more genetically alike because of inbreeding, resulting in more equal biological response. Very specific parameters can be investigated separately and the animal can serve as its own control. However, experiments in animals obviously cannot replace human studies and several limitations from results in animals are present as well. The present investigations in dogs were done in healthy bone with a healing and remodeling capacity much larger than in humans. The remodeling rate in dogs is 2–3 times higher than in healthy humans [220]. In addition, healing capacity in patients might be depressed by factors such as metabolic diseases, drugs, tobacco, alcohol etc. [64,183,196]. In each study age and sex matched dogs were used.

Humans. The opportunity for investigating bone implants in humans in a paired design under very controlled conditions was achieved in study VI. The patients suffered from an acute spinal fracture and were scheduled for operation by internal fixation (pedicle screw implants and rods) and posterolateral fusion. Although the investigation was done in humans several limitations from this study are present. The implants were inserted as non-weight-bearing in healthy bone without access of joint fluid.

Ethical considerations. Animal studies were 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. The human study was approved by the Medical Ethics Committee and informed consent was obtained from each patient before surgery.

Design of studies

The strongest design was used for the purpose given the highest priority. In all studies but study I, the paired design was applied. In study VII, the effects of crystallinity had higher priority than observation-time. Each animal was its own control for crystallinity (paired design) whereas implantation-time was an independent variable. In study I, which was based on samples from an earlier experiment, the immobilized and continuously loaded groups were independent [372]. Random allocation between right and left leg and between cranial and caudal implantation in the iliac crest were done to eliminate differences in weight-bearing pattern and in bone repair rate. Non-implanted control implants were included when loss of Ca-P coatings was estimated. To eliminate bias the treatment groups were blinded until mechanical and histological analyses were done.

Study IV and V were part of the same experiment; moreover study II was conducted in the same dogs. Uncoated titanium implants were not included as controls in study II and III because they would result in fibrous anchorage. In addition, the studies aimed at investigating different Ca-P coatings (IV–VII) and loading conditions on resorption of HA (I) and not on the effect of micromovements on bone ingrowth alone. The study on sampling efficiency was designed to show the need for sampling in implant research and to demonstrate how much work should be done (VIII).

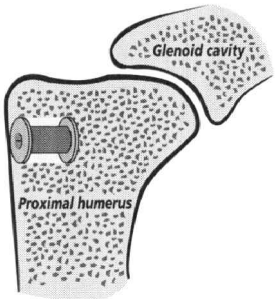


Figure 6. **The gap model in the proximal part of the humerus (Study II).** The implant is inserted into trabecular bone in a stable non-weight-bearing position. Initially, the implant is surrounded by a one-mm gap ensured by a footplate and a washer fixed by a screw.

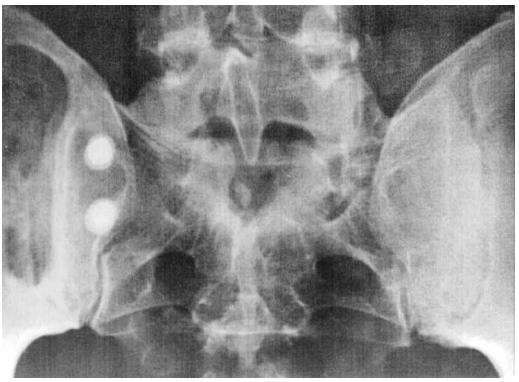


Figure 7. **The gap model in the posterior part of the iliac crest (Study VI).** The X-ray demonstrates that two implants, one hydroxyapatite- and one fluorapatite-coated are inserted.

Implantation periods from 16 weeks to one year were chosen in order to derive answers to specific questions. In studies with implantation periods of 16 weeks (I, III, VII) the early effects were analyzed, whereas the longer-term effects were investigated in 25 weeks, 32 weeks and 13 months studies (II, IV–VII).

In the present series of experiments non-inserted control implants were used as control for loss of coating in vivo. Control implants were treated identically to test implants during preparation of specimens and cut of section. Due to gaps around the implants in all experiments, the coating could not peel off during operation. Consequently, no time-zero group (implants harvested immediately after surgery) was included in any of the studies.

Sample size. The sample size was calculated from the formula [3]:

$$n_1=n_2= 2(t_{2\alpha} + t_{\beta})^2 \times SD^2/D^2$$

Error of first kind (2α), which is the level of significance or the risk of identical results if in fact they differ, was selected to 5%. Based on previous studies, an SD of 50% for both mechanical and histological data seems justified [367]. The minimal clinically relevant difference (D=MIREDIF) not to be overlooked between test groups was selected to 70%. Error of the second kind (β), which is the risk of concluding that two effects are identical if in fact the difference is below the MIREDIF (false negative result), was chosen to be 20%. Based on these assumptions, at least 7 experimental subjects should be included in each experiment. For dog experiments, 7–8 dogs were included (I–V, VII) whereas for the human study 15 patients were included as drop outs were anticipated (VI).

Applied experimental models

To optimize reproducibility, standardized surgical procedures in combination with fluoroscopic control were used for each model. The site for implantation was selected in order to mimic the clinical situation. Therefore, every model applied was situated in trabecular bone. In animal studies implants were inserted into the metaphyseal area of the distal femur or the proximal humerus whereas implants were placed in the iliac crest in humans. The bone in the iliac crist is well characterized and consist of trabecular bone. Because gaps are often present at the interface between the prosthetic component and the surrounding bone, gap models were used [343]. Moreover, weight-bearing intraarticular models were included with or without micromovements. Cavities for the implants were created by using a cannulated hand drill to avoid thermal injury of the surrounding bone. Two, basically different models were applied: A non weight-bearing model (II, VI) and a weight-bearing model (I, III–V, VII).

The non weight-bearing model was used in the proximal humerus in the dog and in the iliac crest of humans (Figure 6 and 7). The non weight-bearing model is a basic experimental model with less "noise" than the weight-bearing model, but with

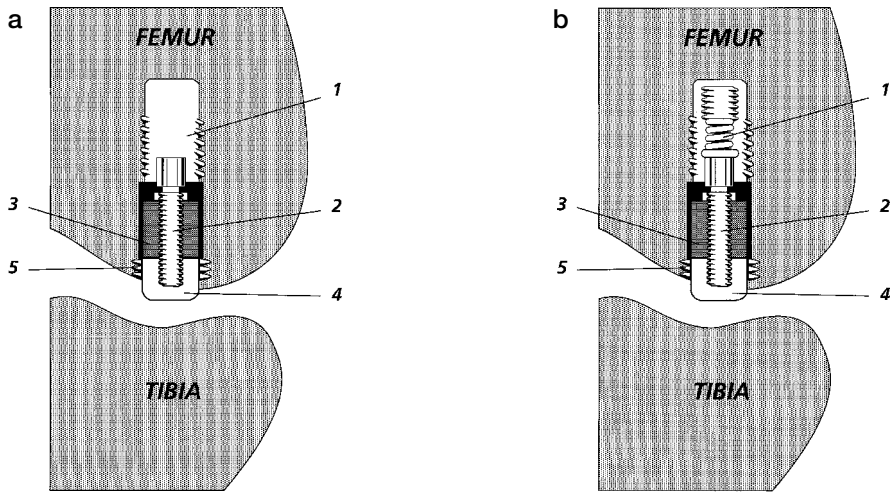


Figure 8. **Schematic drawing of the stable (a) and the dynamic implant device (b) inserted into the medial femoral condyle of the knee.** All the components are made of titanium alloy, with the exception of the polyethylene plug, which is made of ultra high molecular weight polyethylene. The anchorage screw (1) has self-tapping threads to ensure firm fixation into the trabecular bone. In the *stable model (a)* the anchorage screw is empty, and a piston (2) is fixed to the anchorage screw. In the *unstable dynamic model (b)* the anchorage screw contains a spring (1) which is connected to the piston (2). A test implant (3) and a polyethylene plug (4) are mounted on the piston. A ring (5) serves as a bearing and centralizer for the polyethylene plug. The gap around the polyethylene plug and implant communicates with the knee joint space. During loading, the load from the tibial plateau will be transferred to the PE plug and the test implant. Then the plug and the implant will displace axially and the spring will tighten in the *unstable dynamic model (b)*. When the leg is unloaded, the spring will move the implant back to the initial position. A controlled micromotion predetermined to 150 μm, 250 μm or 500 μm will occur at each loading and unloading cycle. The black area represents a 0.75 mm gap. Modified from Søballe et al [375].

less clinical relevance. Implant stability was ensured by a footplate and a top washer. In order to avoid bone bypassing the gap conducted by the washer or the footplate, a model without washers would be preferable. However, our model necessitated their use in order to secure implant stability and an equal gap around the implant.

The weight-bearing model, used in studies I, III–V, and VII, was developed by Søballe et al. [367, 370,372,375]. The implant device was implanted into trabecular bone of the medial femoral condyle as a *stable or unstable system* (Figure 8 A and B). The implant model is clinical relevant with respect to several conditions. First of all, the implant is weight-loading during each gait-cycle, moreover the joint fluid has access to the periimplant gap simulating the clinical situation of implants [344]. The significance of weight-bearing was documented by Søballe et al., who demonstrated that stable weight-loaded implants increased bone ingrowth and mechanical fixation compared with non weight-loaded implants [367]. Weight-loading is also important to prevent bone resorption and to stimulate bone formation and adaptation in accordance with Wolff's law [140,182,420].

The unstable weight-bearing implant system is well-characterized (Figure 8B) and was used in study I, III, and VII [370,374,375]. Micromotion may occur in the clinical situation during each gait cycle and has been demonstrated to be in the range of 100 to 600 μm [40,403]. Consequently, micromovements of 150, 250 and 500 μm were used. Range of micromotion and stiffness of the spring were calibrated before implantation and tested after the implants were harvested. Generally, the stiffness of the spring showed an increased from the preoperative values to autopsy whereas the displacement decreased (Table 3). The presence of micromovement in vivo during the observation period could not be measured directly but fluorochrome labeling during the observation period might be an indirectly method. Labeling done 8 weeks after surgery showed absence of fluorochrome in a circular zone close to the implant surface indicating absence of bone ingrowth at 8 weeks after surgery in all but one implant (I, III, and VII).

Table 3. Test of the micromotion device preoperatively and at autopsy, mean ± SD

	Stiffness (N/mm)		Displacement (μm)	
	Preoperative	At autopsy	Preoperative	At autopsy
Study I	15.7 ± 1.5	22.4 ± 5.5*	167 ± 14	170 ± 33
Study III	14.9 ± 1.3	16.5 ± 2.8*	504 ± 8.7	469 ± 18*
Study VII	16.7 ± 1.5	25.4 ± 15*	240 ± 25	195 ± 49*

*p<0.05

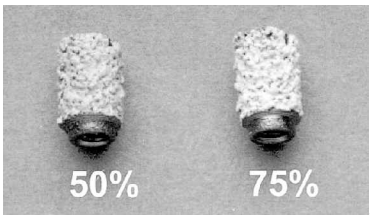


Figure 9. **Porous-coated titanium alloy implants coated with hydroxyapatite (HA).** The HA coatings had crystallinities of 50% and 75%. Note the difference in gray tone levels of the HA coating indicating varying crystallinity.

Implant characteristics

The implants for all studies were manufactured by the same company (Biomet Ltd. USA). Studies II–V had identical batch numbers, and thus identical control implants were included. All implants used were cylindrical in shape to standardize surgical procedures and to optimize mechanical and histological analyzes. The implant size was chosen according to the anatomical proportions at the implantation site [367]. The metal implant was made of titanium alloy consisting of 6% aluminium, 4% vanadium and 90% titanium (Ti-6Al-4V) which later was coated with a Ca-P coating. The decision to use titanium alloy was based on literature review and our previous experiments, which indicate that corrosion, biocompatibility, elastic modulus and bonding strength between HA coating and metal favor the use of titanium alloy (see section on biomaterials). In studies I, IV, V, VII implants with a plasma-sprayed porous coating were used (Figure 9). Comparative studies between grit-blasted and porous-coated implants were carried out in studies II and III, because the majority of Ca-P coated prostheses at that time had a grit-blasted surface [71,133,389]. Finally, study VI included only implants with a grit-blasted surface which enabled us to measure coating thickness and presence of tissue type covering the coating. Implants with both kinds of metal surfaces are used clinically.

Surface topography. Surface topography can be evaluated descriptively by scanning electron microscopy or quantitatively by using a profilemeter. In the present studies, surface roughness was measured by profilemeter measurements by the Institute of Technology (Aarhus/Taastrup, Denmark). A tipped probe provided a profile of the surface during movement. The arithmetic mean value (R_a) or average surface roughness was used as parameter for the surface roughness. R_a is defined as the mean distance from the mean line to the valley and peak points of the surface. 4–6 measurements on each implant were done and a mean value was calculated. R_a was 29–41 μm and 3 μm after application of the Ca-P coating for porous-coated and grit-blasted implants, respectively. Within each study no statistical significant difference between implant types was found.

Calcium phosphate coatings

As coating quality varies between vendors and even between different batch numbers, only one supplier (BioInterfaces Inc, San Diego, CA) was used for the present studies [76]. All coatings were plasma-sprayed using the technique described earlier. The coating qualities are given in Table 4. Quality parameters, apart from coating thickness and porosity, were assessed by the supplier. XRD patterns of FA and HA coatings (II–VI) are presented in Figure 3.The coatings from studies II–VI originated from the same batch number, whereas coatings for studies I and VII were done during separate procedures. In study VII, coating crystallinity was confirmed by an independent laboratory (*Joop Wolke Phd, Department of Biomaterials, University of Nijmegen, Holland*).

Table 4. Characterization of hydroxyapatite (HA) and fluorapatite (FA) coatings, mean ± SD.

Study	Coating, implant surface	Purity (%)	TCP [a] (%)	Ca/P [b]	Crystallinity [b](%)	Thickness (μm)	Porosity (%)
I	HA, porous-coated	100	0	1.64–1.70	75	23 ± 1.1	15
II–V	HA, porous-coated	97	3	1.69	68	45 ± 5.7	15
	HA, grit-blasted	97	3	1.69	68	66 ± 10	15
	FA, porous-coated	97	3	1.61	74	44 ± 5.8	15
VI	HA, grit-blasted	97	3	1.69	68	69 ± 2.1	15
	FA, grit-blasted	97	3	1.61	74	68 ± 2.2	15
VII	HA-50%, porous-coated	97	3	1.69	50	51 ± 2.0	16
	HA-75%, porous-coated	97	3	1.69	75	50 ± 6.0	15

[a] TCP = tricalcium phosphate. [b] Determined by manufacturer.
Crystallinity and purity of the coatings were measured using X-ray diffraction analysis.
Porosity was determined by scanning electron microscopy of the non-inserted control implants using image analysis.
The mechanical properties of the ceramic coating implant interface were determined according to ASTM standards by the manufacturer [5]. The shear strength was 24 MPa and tensile strength 51 MPa.

Postoperative care

Canines. All dogs were allowed full weight bearing, postoperatively, and they were inspected regularly. Animal care was done under identical conditions in individual cages. Out-door activities were allowed every day.

Humans. Postoperatively, the spine was braced for 3 months. The patients were informed not to take nonsteroidal anti-inflammatory drugs during the observation-period of one year to eliminate possible negative effects on bone healing [64,183,196]. Alcohol and tobacco consumption were not registered.

Preparation of specimens

The knees were opened under sterile conditions and cultures were taken from the joint fluid. Several cultures showed colonies of bacteria. This was ascribed to contamination during sampling, because no signs of infection were present neither clinically nor histologically in synovial biopsies.

The implants were harvested and sectioned and specimens for biomechanical and histological analyzes were obtained (Figure 10). Specimens for mechanical testing were always taken from the most distal or superficial part of the implant whereas specimens for histology were taken more

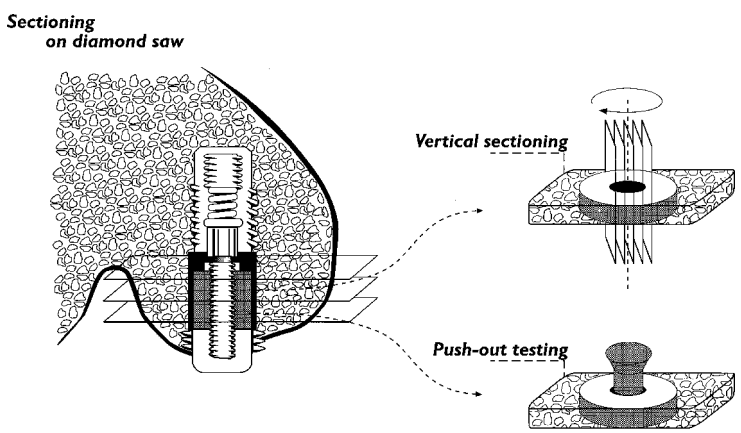


Figure 10. **Preparation of bone-implant specimens**. A water-cooled diamond band saw was used. The first specimen was used for mechanical testing and was stored at –20 °C until testing. The second specimen was used for histological evaluation. It was dehydrated in graded alcohol and subsequently embedded in methylmetacrylate. Later, the specimen was sectioned using the vertical section method (II–VIII).

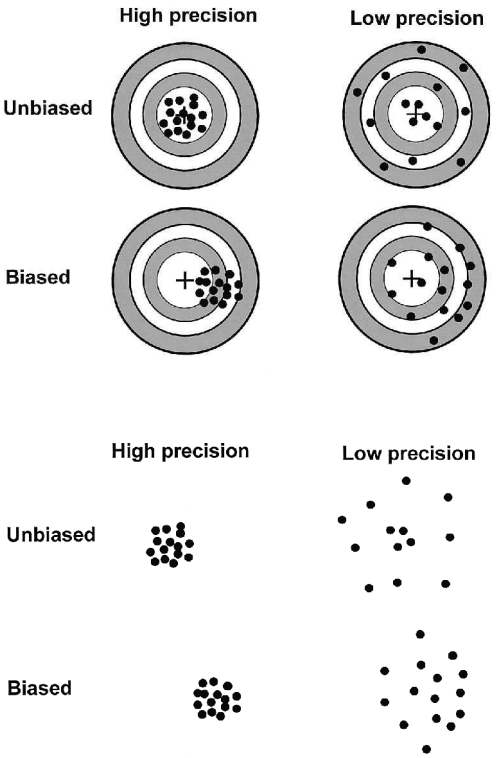


Figure 11. **Schematic drawing showing the significance of precision and biasness.** A) The unbiased and biased estimator with high and low precision are shown. For estimators with both high and low precision, the unbiased estimator converges towards the true value when sampling size is increased. The efficient estimator will go to a stable value after sampling a few items. The biased estimator might have both high and low precision, but without reaching the true value—no matter the sampling size. B) In reality, the true value of the estimator is unknown, illustrated by removal of the targets, i.e. the truth is unknown. This demonstrates that bias is impossible to detect or quantify from the data alone, whereas low or high precision is detectable. Modified from Gundersen [154].

proximally or profoundly. This selection might reduce variance of the estimates but might introduce bias if the area of interest does not represent the entire implant (Figure 11). However, in our studies good correlation between mechanical and histomorphometric results was demonstrated indicating that no bias had occurred. In humans (VI), mechanical testing could not be performed due to limited surrounding bone. The entire implant was embedded and sectioned for histomorphometry.

Mechanical testing

The initial and secondary mechanical fixation of a joint prosthesis is very important for survival of the prosthesis [214,341]. Initial fixation is obtained by press fit insertion of the implant and is crucial for the quality of secondary fixation obtained by the surrounding tissue. In the present series of experiments, mechanical fixation was estimated by push-out testing which is destructive. Other destructive tests such as pull-out and removal torque tests exist [56,145]. They are often used when testing implants with threads such as screws and dental implants. Few authors have advocated tensile testing which might be explained by the fact that very restricted implantation models have to be used [212]. Reversing the test direction from a push-out to pull-out test will provide different results due to the non-uniform distribution of radial interface stresses and due to the non-linear nature of the analysis [352]. Based on finite element analysis of cylindrical implants a torsion test might give a more uniform shear stress distribution and will result in lower shear stresses than a push-out test [106,168]. A push-out test might be easier to standardize than a torque test which however would be relevant to the clinical situation of e.g. THA where high torsion forces are present during weight-bearing.

A disadvantage of destructive tests is that the interface is interrupted which makes histological analysis of the sample impossible. This could be eliminated by using non-destructive tests [18]. In addition, a non-destructive fatigue test might be more clinically relevant, simulating loading below the maximal fixation strength before failure of the interface. However, a fatigue test would require many experimental individuals to test each implant group at different strains and Hz.

Push-out testing

Mechanical fixation was calculated from load deformation curves obtained during push-out testing (Figure 12). The most important parameter for implant anchorage seems to be energy absorption which is a function of interfacial strength and stiffness. Although strength values are equal, energy absorption might differ significantly when the interface is able to deform and absorb more

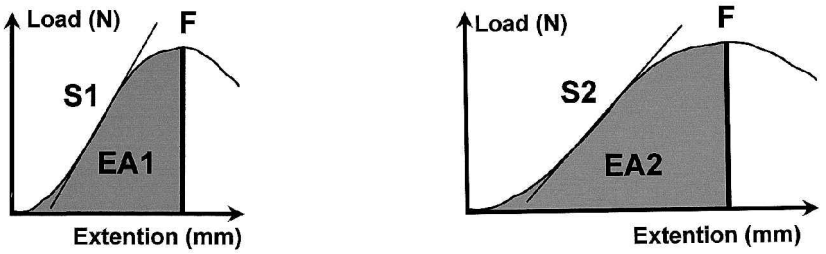


Figure 12. **Load deformation curves obtained during push-out testing.** Ultimate shear strength was determined from the maximum force (F) applied to the implant until failure of the bone implant interface. Apparent shear stiffness (S) was calculated from the slope of the load displacement curve. Energy absorption (EA) was determined as the area under the curve until failure (blue). Although the load deformation curve showed equal strength values (F) EA2 was significantly higher than EA1 due lower stiffness (S2) of the interface.

energy due to different interface stiffness (II, III). Low stiffness of the porous interface expresses a less brittle interface allowing more strain to occur before failure. During testing, both mechanical interlock and true interface bonding between tissue and implant surface will contribute to implant fixation of porous-coated implants, whereas the grit-blasted implant surface is without interlocking. Several authors have suggested that push-out tests are only valid under very restricted testing conditions, as outlined in Table 5.

Table 5. Factors affecting results of push-out testing [19,75,76,95,168,351,352]. The factors are essential when comparing results from different studies.

Study design
Implantation site
Bone type
Observation time
Implant fit
Load-bearing conditions
Mechanical stability
Implant type
Geometry and dimension
Surface texture: polished, grit-blasted, porous-coated
Composition: bulk or compositional material
Preparation of specimen
Thickness
Storage: fresh, frozen, formalin fixed.
Test conditions
Calibration of test apparatus and x-y recorder
Alignment and orientation of specimen (push-out direction)
Support jig: clearance of the hole
Load displacement rate

We used implants with identical geometry and dimensions in all studies and the effect of a non-uniform stress distribution along the implant interface was reduced by including bone-implant specimens with similar thickness [18]. Thickness varied from 3 to 3.5 mm with very low variation within each study. In addition, push-out data were normalized by the surface area of each implant specimen tested. Specimens were frozen until testing which might have influenced the bone-implant interface. However, mechanical properties of trabecular bone do not change after freezing for 100 days and neither after repeated thawing and freezing [250]. By contrast, formalin-fixed specimens seems to produce higher shear strength values [134].

Regarding test conditions, clearance of the hole is a critical parameter as stated from finite element analyses [95,168]. Clearance is the distance between implant periphery and the support jig on the test machine. Dhert et al. calculated that a very tight fit of 0.1 and 0.3mm resulted in high stresses at the site where the jig edge supported the bone, whereas clearance values of 0.5, 0.7 and 1mm had low stress peaks. In our studies, the clearance was 0.5mm. Load deformation curves were obtained by an X-Y plotter and data were recorded simultaneously on a computer (Figure 12). For load-bearing studies (I, III, IV, VII), a push out direction equal to the load transfer direction was chosen. Variation in test conditions was minimized by testing all specimens from one study the same day without changing the setup. *Evaluation of failure site.* During push-out testing the entire bone-implant interface complex is tested and differentia-

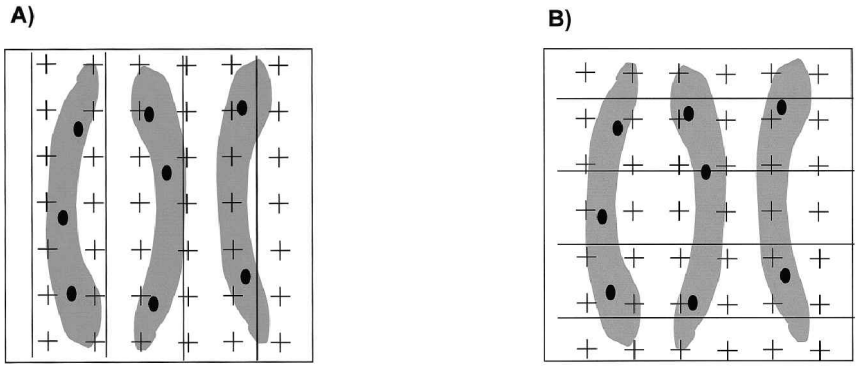


Figure 13. **Schematic drawing illustrating the significance of probe orientation when estimating bone surfaces.** In a) 6 intersections with bone surfaces are counted, whereas 24 intersections are counted in b) resulting in a 4-fold higher surface area although the probe density was identical. In contrast, volume density calculated as number of hits on bone divided by the total number of points per area evaluated was equal (12/42). This demonstrates that estimation of surface area of anisotropic structures is sensitive to orientation of the probe whereas estimation of volume densities is independent of the orientation.

tion between implant-coating and coating-bone stresses is impossible. However, the failure site indicate which interface that contributes to the fixation strength. In the present study, the failure site was evaluated by stereo microscopy at a magnification of x 15–25. Distinction between a "naked" metal implant surface and a surface with tissue or coating was in most implants obvious and was confirmed by light microscopic evaluation in selected cases.

Histological and histomorphometric evaluation

Histological evaluation was done by simple description (qualitatively) of the histology and by histomorphometry (quantitatively). Conventional histomorphometric evaluation of bone-implants has traditionally been made on transverse sections without considering the orientation of the surrounding tissue [68,363,372]. For estimation of volumes, this raises no problems. However, to avoid getting biased information of surfaces, trabecular bone and implant surface must be assumed to show isotropic orientation, i.e. without any preferred orientation [7,155,402,406]. With respect to trabecular bone it has been shown that morphology and mechanical properties are orientated, i.e. anisotropic conditions exists [128,395,410]. Moreover, when inserting a loaded implant, the surrounding bone will adapt to the new situation by orientating the bone trabeculae [140,399]. Therefore, to have unbiased estimates of surfaces, either isotropic uniform random (IUR) sections or IUR test lines on vertical sections are mandatory [7,402] (Figure 13). These requirements could be fulfilled by application of stereological methods. However, in study I, only one cross section from each implant was evaluated by scanning electron microscopy. It was assumed that the implant surface was isotropic. For estimation of bone remodeling in studies III and IV, cross sections were included due to the thin bone blocks because a vertical axis would have failed for evaluation of bone remodeling.

Stereology

By using stereological methods, quantitative information about three-dimensional structures could be obtained from observations made on two-dimensional sections [155]. The sections contain only information of three-dimensional structures in a statistical sense and for this to be true, sampling of sections must be uniform and random which ensure *unbiasedness*. An optimum sampling procedure requires planning of the sampling method before processing of specimens and sections, in order to have an *efficient* method and in order to get *unbiased* estimates. The concepts of *efficiency* and *unbiasedness* mean with a low variability after spending a moderate amount of time, and without systematic deviation from the true value, respectively [155,157]. An efficient procedure must be systematic. Bias might be present at dif-

ferent levels, e.g. during sampling of sections or at the estimator level produced by the analysis method. Bias is a very important factor to eliminate whereas precision is a more relative value (Figure 11).

Efficiency of systematic sampling

By the introduction of a new hard-tissue saw which was able to perform numerous sections from each bone-implant specimen, the importance of sampling efficiency became more evident than previously. Sampling efficiency and biological variation have been investigated on bone remodeling data and in the present study in bone-implant research [153,400,401] (VIII). The variance observed in the study group is the sum of variances from a hierarchy of sampling levels [158,159,276,309] (Figure 14):

1) Variance among humans within group
2) Variance among sections within each implant
3) Variance among sides of the implant within sections
4) Variance among fields of view within each section
5) *Variance due to grid counting (position and orientation)*

Only the sampling items at the top level, humans, are natural items; the lower levels are all artificial sampling items. From each sampling level, contributions to the total observed variance were calculated as:

$$ObsCV^2 = CV_h^2 + CE_{is}^2 + CE_s^2 + CE_f^2$$

where CV_h is the true biological variance, CV_{is} is variance between implant sides, CE_s is variance between sections, and CE_f is variance between fields of view. The true biological variance cannot be calculated directly, but by rearranging the equation above.

Efficiency of systematic sampling was evaluated by estimation of variance in study VIII. The basic sampling design consisted of five implants from five humans (study VI), 14 sections per implant, and of 10 successive adjacent fields of view on each implants side, i.e. 20 fields of view per section which were reduced successively (Figure 14). The analyses showed that the variance of bone ingrowth at the top level of sampling, humans, varied around 20% also when sampling was

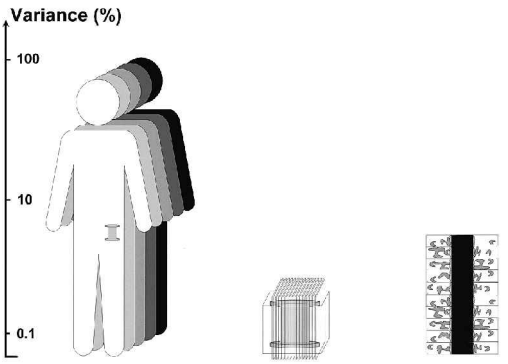


Figure 14. **The sampling hierarchy and contributions to the total observed variance shown as icons of humans, sections and fields of view.** The Y-axis (logarithmic scale) expresses variance in percentage of the total variance. The total observed variance was contributed by variance among five humans, 14 sections, and 20 fields of view. Variance from implant sides is not shown. Most important for the total variance of a specific parameter was the true biological variance among humans, whereas variance from sections, fields of view and number of items counted were so low as to necessitate the use of a logarithmic Y-axis.

reduced. At the section level, variance of bone ingrowth between sections increased 4-fold when the number of sections was reduced from 14 to 3-4 per implant. However, because 3–4 sections were evaluated per implant the contribution to the total observed variance was low. The major contribution to the total variance was the true biological variation between humans, which varied from 60 to 100%, whereas variance from section and fields of view only contributed to a minor degree, in agreement with other biological studies (Table 6) [159,400]. The contribution from probe position and orientation, included in the variance from fields of view, was negligible due to application of 280-1680 lines per implant. By optimizing the sampling design work load could be reduced significantly without changing the quality of the data which still had low variance and were unbiased. The work load at the hard-tissue saw could be reduced to less than 25% and at the microscope to less than 10% (VIII). Given that enough sections are sampled and that sampling is done systematically, uniformly and randomly and that sampled items are approximately independent, some simple rules determine the variance. By sampling 100–200 independent hits by the probe the contri-

Table 6. Contributions to total observed variance (ObsCV) [a] between histomorphometric data from humans (n=5) (%)

Sampling intensity	Humans	Implant	Sections sides	Fields
Basic design: 14 sections, both implant sides, all fields of view				
S (Bone ingrowth)	99.9	0	0.1	0
V (Fibrous tissue)	100	0	0	0
Basic design reduced to one implant side and 1/3 fields of view				
S (Bone ingrowth)	77.6	9.1	0.1	13.2
V (Fibrous tissue)	59.9	37.1	0.1	2.9
Basic design reduced to one implant side and 1/3 fields of view and 50% probe density				
S (Bone ingrowth)	77.4	9.0	0.1	13.5
V (Fibrous tissue)	57.2	33.2	0	9.6

[a] The ObsCV consists of the true biological variance between humans (Cv_h), variance from implant sides (CE_{IS}), variance from sections (CE_s), and the variance from fields of view (CE_f).
S = surface, V = volume.

bution to the total variance from fields of view will be 7–10% (CE=1/√n), which for most experiments is significantly lower than the biological variation. Independency is present when the distance between sampling items (sections, fields, lines or points) is sufficiently large.

Vertical section method

The vertical section method was applied in studies II–VIII. It was developed to solve a stereological problem in anisotropic structures, namely the estimation of surface area density [7]. The method was introduced by Vesterby et al. to bone biopsies and later by us to bone implant research [300,402]. Four requirements must be fulfilled in order to obtain unbiased estimates of surface density (Figure 15). The vertical axis is defined as perpendicular to a given arbitrary "horizontal" plane, which must be free to rotate about; thus a cylindrical implant with a set of sections parallel to the long axis is suitable, whereas a transverse section of a cylindrical implant is not a vertical section. Suitable straight test lines or a system of cycloids on the monitor is provided by a software program (CAST-Grid®, Olympus Denmark A/S, Albertslund, Denmark) (Figure 16).

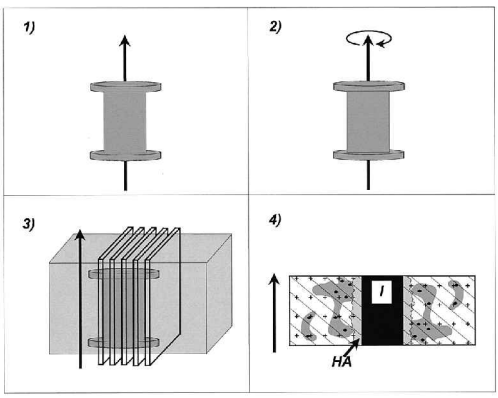


Figure 15. **A schematic drawing demonstrating the concept of the vertical section method.** Each part represents one of four requirements which must be fulfilled for the vertical section method. The icons illustrate an implant (1) which is rotated around the vertical axis (2), subsequently embedded and sectioned (3) and finally the sections are analyzed in the microscope with test systems which are 2D anisotropic with respect to the observable axis of specimen rotation (4).
Requirements: 1) The vertical axis must be defined. All sections must be parallel to the vertical axis. 2) Random rotation of the section plane must be achieved by rotating the specimen around the vertical axis. 3) Random positioning of the sections must be achieved by uniformly random sampling, preferentially systematic, and then serially cut sections are performed parallel with the vertical axis. 4) On the vertical section, the vertical direction is identified in each section and a set of test lines is applied in the microscopic field of vision. The lines must be isotropic and uniformly random in 3D space. Mathematically, this requires a 2D test line which is given a weight proportional to the sine of the angle between the test line and the vertical axis or it require application of cycloids. For this reason the direction of the axis must be known in each section. Further details and mathematical explanations have been published previously [7,402]. Suitable straight test lines or a system of cycloids is provided by a software program (CAST-Grid®). I=implant, HA=hydroxyapatite.

Deviation from the defined vertical axis might be a methodological problem in the vertical section method. This occurs when the saw axis is not perfectly parallel with the long axis of the implant and the deviation might be a few degrees. Mathematically, the method is robust to deviations from the true vertical axis, however, the accurate figures are not yet known. Another problem in the vertical section is estimation of volume densities in the gap. In practice, given that 14 sections are obtained from the implant with a diameter of 6 mm, then two sections will be cut 2.3 to 3 mm from the implant centre. For these "tangential" sections, the 2D gap size will increase 2–3-fold.

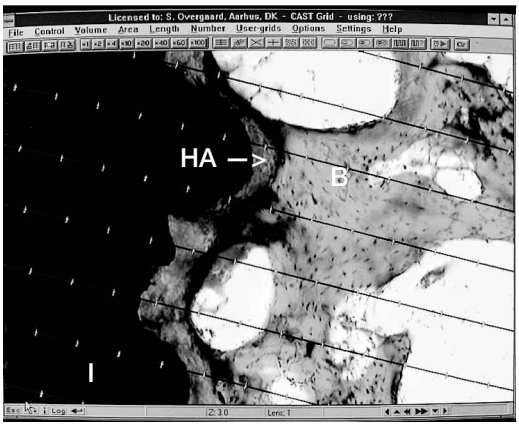


Figure 16. **Photo of the monitor.** The field of view transferred from the light microscope. Sine weighted straight test lines (blue) with points (white) are provided by a software program (CAST-Grid®, Olympus Denmark A/S, Albertslund, Denmark). Intersections with surfaces will provide surface area whereas points will provide volumes. B=Bone, I=implant, HA= hydroxyapatite coating.

Therefore, to reduce bias of bone volume densities, estimation was done on cross sections in studies III, IV whereas only the more central sections were used for bone volume estimation in studies II, VI, and VII.

Preparation of undecalcified sections

Before preparation of sections, the sampling design for the specific study was determined in order to optimize embedding and sectioning procedures (II–VII). Undecalcified sections for SEM and LM were done with the implant in situ. Other methods remove the implant after decalcification or by electropolishing [386]. Since these procedures may disturb the interface we used methods with the implant in situ. One drawback of methods with the implant in situ is that thicker sections are needed.

During this series of experiments the method for preparing sections for LM was changed from the grinding and polishing method (II,IV,V) to a recently developed sawing technique (III, VI, VII, VIII) [98,232]. The advantages of the new sawing technique are that 1) sections are performed in one step, 2) sections can be cut serially with precise distance between sections, 3) the thickness of the section can be adjusted from 10 μm, and 4) each section can be done quickly and safely within 10–15 minutes. By contrast, the grinding and polishing processes are very time-consuming, thicker sections are produced and the distance between sections is not precise. We have operated with a section thickness of 50 μm for the grinding process in order not to loose the metal implant. and sections with a thickness of 25 or 50 μm were obtained from the sawing technique. Thinner sections would have been more optimal, however, several attempts were done to reduce the drawback of the "thick" sections [209]. First of all, a bone surface staining method (Light green) was used, which has a penetration depth of 5–10 μm [144] (VII). Furthermore, the microscope was equipped with a lens reducing the thickness of the focus plane (10x/0.40, ∞/0.17). Finally, during evaluation only one focus plane was analyzed. Preparation of sections without implants for TEM and for evaluation of bone remodeling was done using standard methods [213,269].

Histomorphometry

Several evaluation methods are used for histomorphometry. Sumner et al compared SEM, LM and microradiography. They found good correlation between measurements made by SEM and LM, while microradiography estimators were biased overestimating bone ingrowth and underestimating porosity [359]. We have used LM and SEM in the present series of studies. Advantages of SEM in back scatter mode are that the images have very high resolution and that only a few μm of the implant surface are analysed during scanning [27,28, 186,335,336,353,392]. However, bone marrow and fibrous tissue could not be distinguished by the available SEM. In addition, it was impossible to transfer the image from the SEM to a PC monitor which is why we chose to continue the following studies by using LM. The advantages of LM is that the procedure is fast and that the field of view can be transferred to a monitor for application of specially designed grid systems. One drawback of transferring the field of view is loss of resolution from the LM via the videocamera to the monitor. However, we found the resolution of LM satisfactory, and bone and Ca-P coating were easy to distinguish (Figure 16).

Histomorphometry was evaluated by the linear intercept technique and point counting in all studies with the exception of VI, where coating thickness was measured directly on the monitor

[155,157,221]. Linear intercept technique and point counting seems to be superior to semi- automated image-analyzed based methods because image-based systems are time-consuming and because they do not reduce the total observed variance significantly [156,264]. Statistically, the use of image analyzed methods can only reduce variance at the lowest level (point or intersection counting) (Figure 14). However, this seems of minor importance when systematic unbiased sampling is used because the lowest level contributes only very little to the total observed variance in the study group (VIII). Potential pitfalls must also be taken into consideration when using image-analyzed methods. For SEM, adjustment of grey level thresholds are important in order not to over- or underestimate bone volume, whereas LM with image analyzes of colors is even more complicated and often manual corrections are needed. The importance of basic sampling requirements are often overlooked when investigators have access to semi- or full-automated image analysis systems, and inter-method variation between manual and semi-automated methods is significant [356,424].

Resorption parameters of calcium phosphate coatings. Several different parameters for loss of Ca-P coating in vivo were included: HA coverage, surface area, volume and thickness. Coverage is the coating surface area in percentage of the total implant surface. Briefly, surface area per implant length for any surface was calculated as S_L:

$$S_L = [2 \times \Sigma I \times t] / [[l/a] \times L(\text{implant})] \ (\text{mm}^2/\text{mm}),$$

where ΣI is the number of intersections with the surface, t is the distance between sections evaluated, l /a is test line length per area on the monitor, and L is the implant length.

Volume of the HA coating per implant length was estimated as V_L:

$$V_L(\text{coating}) = [\Sigma P(\text{coating}) \times [a/p] \times t] / L(\text{implant}) \ (\text{mm}^3/\text{mm}),$$

where ΣP is the number of points which hit the coating, and a/p is the area per point of the test system corrected for magnification. The thickness (T) of the HA coating was calculated as

$$T = V_L / S_L \quad (\mu\text{m})$$

Coverage alone does not express anything about volume and thickness of the coating. Thus, if the coating only delaminates then coverage would decrease whereas thickness would be unchanged. In addition, if the coating is resorbed uniformly the coverage could be equal to control implants despite significant resorption of the coating.

Coating delamination and particles. Coating delamination and release of particles might occur during processing of sections. Delamination would leave empty gaps at the metal implant interface and was observed occasionally in the present series of studies. However, it might be difficult to determine whether particles are released in vivo or during processing at the LM level.

Alternatives to histomorphometry

It would be of interest to monitor the coating loss in vivo. Scintigraphic evaluation is a method to be considered. This has been done for bulk materials by strontium labeling of HA and TCP [331]. The authors concluded that the method was sensitive, however no correlation with histology was done. Radioactive marked calcium has also been used to estimate loss of Ca-P coatings during implantation but without success. Currently, the only applicable method for estimating loss of Ca-P coatings is histomorphometry.

Evaluation of intracellular deposits of crystals and of bone mineral

Electron energy loss spectroscopy and electron spectroscopic imaging were performed using a transmission electron microscope in order to analyse intracellular deposits of crystals. Thin unstained sections from study V were studied. Controls of HA crystals also embedded in Epon were included. Theoretically, crystals could be displaced into cells during processing of sections but due to well-preserved intracellular structures on serially cut sections this seems unlikely for the included sections.

Fluorine, calcium, and phosphorus content of bone trabeculae were analyzed using a scanning electron microscope equipped with a microprobe with a focal spot of 2 μm in diameter (IV). Each sample was analyzed twice in 2 different regions

at each side of the implant. The distances between analysis points varied between 20 μm and 30 μm. Approximately 50 points were measured per section per zone and the average value was calculated.

Reproducibility

Reproducibility (intra-observer variation) was calculated from double measurements performed by the same person on identical equipment and was calculated as coefficient of variation (CV). The results of double measurements depend on time intervals between measurements. Short-term reproducibility, within one month, will reduce the CV compared to long-term reproducibility after one year [170].

Mechanical testing. Reproducibility (short-term) of energy absorption from the curves printed by the x-y recorder was done on 7 randomly selected curves from study VII and CV was calculated to be 1.4%.

Histomorphometry. Double measurements (approximately 12 months apart) were done on 10 randomly selected implants. The figures for bone ingrowth, surface area of HA, HA coverage and thickness were 12%, 8%, 8% and 19%, respectively, which are acceptable according to earlier studies on iliac crest biopsies [63,316]. No double measurements on bone remodeling and mineral contents were done. Double measurements on bone remodeling parameters have shown CV values between 5 and 20% [63,170,316].

Statistical analyses

Experiences from earlier studies have shown that mechanical and histomorphometric data most often are normally distributed. In every experiment probability plots were done. For normally distributed data, parametric tests were applied; otherwise, non-parametric tests were used. Accordingly, when comparing two groups a T-test was carried out for normally distributed data whereas non-parametric tests, Wilcoxon or Mann-Whitney, were used for paired and independent data, respectively. When comparing three groups, a one-way analysis of variance (ANOVA) on ranks was applied, and pairwise multiple comparison procedures were done by the Student-Newman-Keuls or the Bonferroni method. $P < 0.05$ (two-tailed) was considered significant.

The intra-individual variation on double measurements was calculated as coefficient of variation (CV) by the method described by Therkelsen [383]:

$$s^2 = (1/2k) \Sigma d^2,$$

where k is the number of double measurements and d is the difference between first and second assessment. Then CV was calculated as

$$CV = s/\bar{x},$$

where $\bar{x}$ is the mean value of first and second assessment.

In the study on sampling efficiency (VIII), estimation of the total observed variance (ObsVar) was based on the fact that it consisted of the true biological variance between humans (Var_h) and the sum of variances of the estimators (Var_e) (section (s), implant side (is), and field of view (f)) [158,159,276,309]:

$$ObsVar = Var_h + Var_e \quad (1)$$

At the uppermost sampling level, humans, the squared coefficient of variation (CV^2) was calculated whereas contributions to the variance at the other levels was calculated as squared coefficients of error (CE^2):

$$CV = SD(x) / \bar{x} = \sqrt{var} / \bar{x} \quad \text{and}$$
$$CE = SEM/\bar{x} = CV/\sqrt{n}, \quad (2)$$

where SD = standard deviation, SEM is the standard error of the mean, and n is the number of independent sampling items. Then contributions from each sampling level to ObsCV were calculated from:

$$ObsCV^2 = CV_h^2 + CE_{is}^2 + CE_s^2 + CE_f^2 \quad (3)$$

The contribution from variance between sections was calculated as [159]:

$$CE^2 = (3A - 4B + C)/240/sum^2 \quad (4)$$

which was based on the actual data set.

Variance between fields of view was calculated as CE^2 from Eqs. 5 and 6. The prediction of the

contribution from a certain sampling level was influenced by the sampling fraction (S.F.) and was calculated as [157,158]:

$$CE^2 = (1 - S.F.) \times CV^2 / n \quad \text{for independent data} \quad (5),$$

and as

$$CE^2 = (1 - S.F.) \times CV^2 / n^4 \quad \text{for non-independent data} \quad (6)$$

In the basic sampling design, S.F. ranged from zero (eg. the 14 sections were sampled from an infinite number of possible 2D section planes through the 3D specimen) to 1 when all of both sides of the implant are studied on the sections. Sections and fields of view were non-independent data, whereas humans were considered independent data. Moreover, every third field of view was considered independent data because of the distance between the fields of interest at the present magnification.

Results of own studies

Mechanical fixation and bone ingrowth

Effects of surface texture: porous coating versus grit-blasted surface. During non-weight-bearing and weight-bearing conditions mechanical testing showed that energy absorption for porous-coated implants was increased 2–3 fold compared with grit-blasted implants, whereas shear stiffness was lower for porous-coated implants (Figure 17) (II, III). By contrast, ultimate shear strength was at the same level for both implant types. Mechanical fixation was higher for implants inserted into the distal femur compared with the proximal humerus although the observation period was shorter (II, IV). This might be explained by higher bone density in the distal femur, and moreover, weight-loading might have contributed to enhanced fixation.

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 (Figure 18). Porous-coated implants had small areas with failure at the metal implant surface on top of the titanium porous coating. Histology showed that all implants in study II had bridges of bone in direct contact with the implant surface, whereas three grit-blasted and three porous-coated implants from study III were surrounded by fibrous tissue with islands of fibrocartilage (Figure 19). This indicates that micromovements of 500 μm approach the limit of HA to conduct bone formation. Bone ongrowth to grit-blasted implants was characterized by thin bone trabeculae on the coating surface whereas bone ingrowth to porous-coated implant was more abrupt (II, III, VI) (Figure 20). Grit-blasted implants had greater bone ingrowth compared with porous-coated implants indicating different surface activities on the implants (Table 7).

Effects of HA versus FA. During stable weight-bearing conditions no difference between HA- and FA-coated implants in mechanical fixation and bone ingrowth were demonstrated after 25 weeks (Table 8) (IV). In addition, no difference in bone volume and bone remodeling in the initial gap was shown (Study IV). In humans, however, HA-coated implants had 30% more bone ongrowth than FA-coated implants ($p<0.05$) (VI).

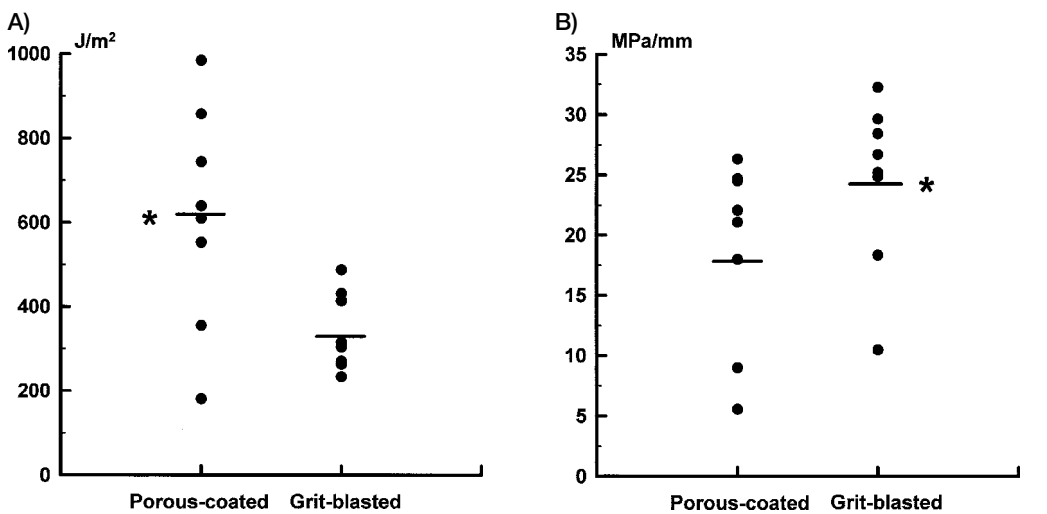


Figure 17. **Push-out test of porous-coated versus grit-blasted hydroxyapatite-coated implants inserted for 25 weeks in a non weight-bearing model (Study II).** Energy absorption (A) was greater and shear stiffness (B) was lower for porous-coated compared with grit- blasted implants. Solid lines represent mean values, *p<0.05.

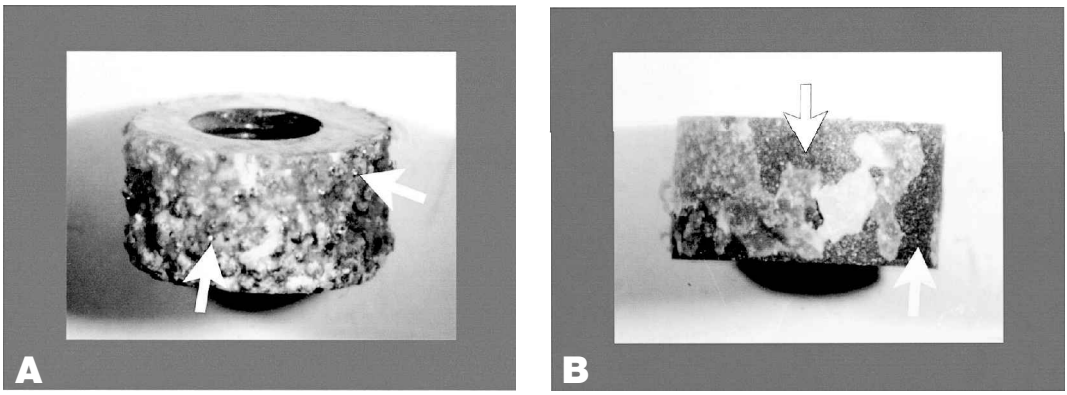


Figure 18. **Macroscopic evaluation after push-out test of porous-coated and grit-blasted implants inserted for 25 weeks (Study II).** A) Porous-coated implants predominantly failed at the hydroxyapatite (HA)-tissue interface. Delamination of the HA coating might have occurred on top of the titanium porous coating (arrows). In contrast Grit-blasted implants B) had pronounced delamination of the HA coating (arrows).

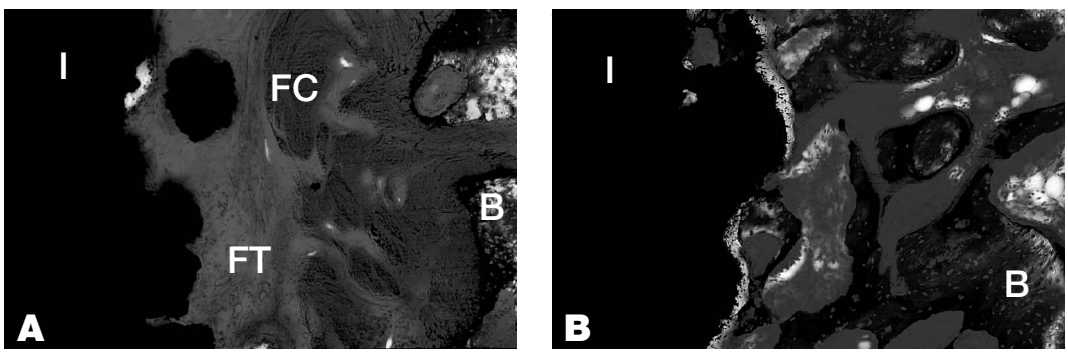


Figure 19. **Histological sections from hydroxyapatite (HA) coated implants subjected to controlled micromovements of 500 μm (Study III).** A) Porous-coated implant surrounded by fibrous tissue (FT) with islands of fibrocartilage (FC). Coverage and thickness of the HA coating (arrow) were considerably reduced compared with B) bony anchored implant. B = Bone. Vertical section, light microscopy, sections stained with light green and basic fuchsin. Original magnification 100x.

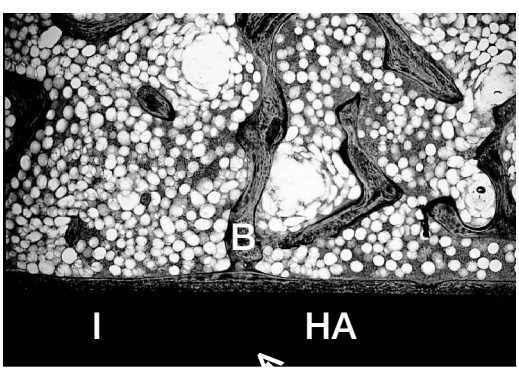


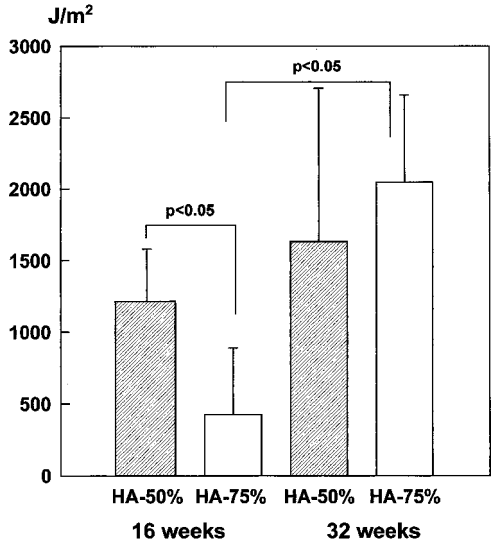
Figure 20. **Histological section from hydroxyapatite (HA) coated implant (I) with grit-blasted surface (Study VI).** Bone ongrowth (B) to grit-blasted implants was characterized by thin bone trabeculae on the coating surface. Light microscopy, sections stained with light green and basic fuchsin. Original magnification x100.

Table 7. Bone ingrowth/ongrowth to porous-coated and grit-blasted hydroxyapatite-coated implants in percentage of the total implant surface, mean ± SD

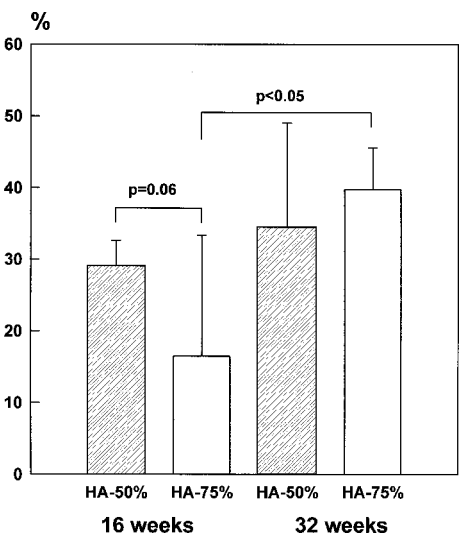
	Porous-coated	Grit-blasted	
Study II (n=8)	48.8 ± 9.1	66.2 ± 11.3	p<0.01
Study III (n=5)	30.1 ± 13.5	44.5 ± 40.4	NS

Table 8. Bone ingrowth/ongrowth to hydroxyapatite- and fluorapatite-coated implants in percentage of the total implant surface, mean ± SD

	Hydroxyapatite	Fluorapatite	
Study IV (n=8)	60 ± 8.4	60 ± 7.7	NS
Study VI (n=13/14)	79 ± 11.1	61 ± 9.8	p<0.001



A) Energy absorption (J/m²). Energy absorption for HA-50% was increased 3 fold as compared with HA-75% after 16 weeks. After 32 weeks, no difference between HA-50% and HA-75% was shown. HA-75% gained stronger anchorage from 16 to 32 weeks, whereas HA- 50% showed no difference from 16 to 32 weeks.



B) Bone ingrowth (%). Bone ingrowth to HA-50% implants was increased 2 fold as compared with HA-75% after 16 weeks. Bone ingrowth to HA-75% implants increased from 16 to 32 weeks while no difference for HA-50% was shown.

Figure 21. Mechanical and histomorphometric results of implants with hydroxyapatite crystallinities of 50% (HA-50%) and 75% (HA-75%) (Study VII). Mean, error bar=SD.

Effects of HA coating crystallinity: 50% (HA-50%) versus 75% (HA-75%) (VII). After 16 weeks during controlled micromotion of 250 μm push-out testing showed that ultimate shear strength and apparent shear stiffness were increased 2 fold for HA-50% whereas energy absorption was increased 3 fold compared with HA-75% (Figure 21a). After 32 weeks no difference in mechanical fixation was found. Histology revealed that after 16 weeks, all HA-50% implants and only four HA-75% implants had bony anchorage whereas after 32 weeks all implants had bone ingrowth. Bone ingrowth to HA-50% implants was increased 2 fold as compared with HA-75% after 16 weeks whereas no difference was shown after 32 weeks (Figure 21b). Thus early bone ingrowth seems to be accelerated by low HA crystallinity.

Loss of calcium phosphate coating in vivo

Porous-coated versus grit-blasted surface texture. Porous-coated and grit-blasted implants had significant loss of the HA coating compared with non-inserted control implants during both non-weight-bearing and a weight-bearing conditions (Table 9) (II,III). Porous-coated implants had more reduction of coating coverage than grit-blasted implants. This might be explained by uneven coating thickness on the porous-coated implants; moreover the micro-environment might have been different around porous-coated and grit-blasted implants.

HA versus FA coating. Both in dogs and humans, significant loss of HA and FA coatings was found (V, VI). In dogs, a tendency towards greater loss of the HA coating was shown but not significantly (V) (Table 9). In humans there was no difference in overall coating loss between HA and FA, however, the HA coating was significantly thinner than FA when bone marrow was present (Table 9) (Figure 22) (VI). Interestingly, the HA coating was significantly thicker than FA when bone was present on the coating surface (VI). This suggests that resorption of Ca-P coatings is governed by multiple factors in the local micro-environment.

Table 9. Loss of hydroxyapatite (HA) and fluorapatite (FA) coatings in vivo, mean percentage ± SD

		Reduction in HA coverage	Reduction in HA thickness
Study I: Weight-bearing implants with micro-movements of 150 μm during 16 weeks			
Immobilized		26 ± 8.1 [a]	33 ± 9.7
Continuously weight-loaded		46 ± 17	34 ± 17
Study II: Non weight-bearing implants during 25 wks			
Porous-coated		6.3 ± 2.8 [b]	32 ± 9.8
Grit-blasted		0.5 ± 0.7	32 ± 14
Study III: Weight-bearing implants with micro-movements of 500 μm during 16 weeks			
Porous-coated			
Bony anchored		47 ± 8.6 [b, c]	43 ± 11
Fibrous anchored		60 ± 2.9	34 ± 2.7
Grit-blasted			
Bony anchored		4.1 ± 4.3	43 ± 15
Fibrous anchored		48 ± 17	55 ± 15
Study V: Stable weight-bearing implants during 25 weeks			
HA		19 ± 4.3	35 ± 7.7
FA		13 ± 7.4	28 ± 8.5
Study VI: Non-weight-bearing implants during 1 year			
HA	Overall	[d]	18 ± 7.4
	Bone ingrowth	[d]	9.9 ± 3.2 [e, f]
	Bone marrow ingrowth	[d]	43 ± 14 [f]
FA	Overall	[d]	17 ± 2.8
	Bone ingrowth	[d]	14 ± 3.2 [e]
	Bone marrow ingrowth	[d]	21 ± 3.5
Study VII: Weight-bearing implants with micromovements of 250 μm			
16 weeks	HA-50%	43 ± 16 [g]	67 ± 9.4 [g]
	HA-75%	20 ± 8.2	49 ± 6.9
32 weeks	HA-50%	35 ± 15 [g]	68 ± 14 [g]
	HA-75%	15 ± 9.4	48 ± 13

Reduction in coverage was calculated as control - test (%) Reduction in thickness was calculated as (control-test)/ control x 100%.
In every study, significant coating loss compared with control implants was shown ($p<0.05$).
Study I: [a] Significant difference between continuously weight-loaded and immobilized implants.
Study II and III: [b] Significant difference between bony and fibrous anchored porous-coated implants.
[c] Significant difference between bony anchored porous-coated and grit-blasted implants.
Study V: No difference between HA- and FA-coated implants.
Study VI: [d] Seven HA-coated implants had small areas with complete resorption of the coating which were covered by bone marrow or bone, however not quantified. No FA-coated implants had complete loss of the entire coating thickness.
[e] Significant difference in coating thickness between HA-bone and HA-bone marrow and between FA- bone and FA-bone marrow.
[f] Significant difference in coating thickness between HA-bone and FA-bone and between HA-bone marrow and FA-bone marrow.
Study VII: [g] Significant difference between HA-50% and HA-75%.

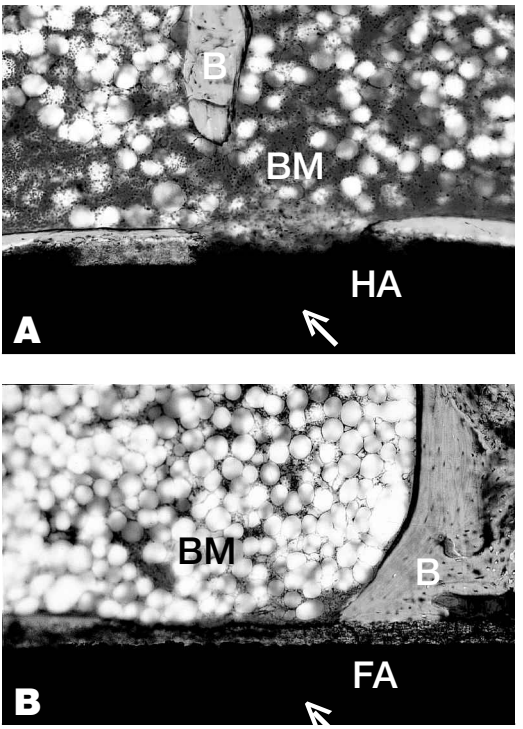


Figure 22. **Histological sections from A) hydroxyapatite- (HA) and B) fluorapatite- (FA) coated implants (Study VI).** The HA coating is thinner than FA in the presence of bone marrow (BM) on the coating surface (arrows) indicating that FA is more stable than HA. B=Bone. Light microscopy, sections stained with light green and basic fuchsin. Original magnification x100.

Transmission electron microscopic evaluation showed aggregates of apatite crystals from the HA and FA coating in direct contact with bone trabeculae (V). Several cell types contained apatite crystals, as verified by element analysis (Figure 23). Thus, crystal fragments were demonstrated in multinucleated osteoclast-like cells, mononuclear and macrophage-like cells and fibroblasts cytoplasmic vacuoles suggesting a role in resorption of Ca-P coatings. Surprisingly, a few osteocytes had crystal fragments in the cytoplasm most likely incorporated in an early stage of cell differentiation.

Low (HA-50%) versus high (HA-75%) HA coating crystallinity (VII). During controlled micromovements of 250 μm both HA-50%and HA-75% coatings were significantly reduced compared with non-inserted control implants. HA coverage and thickness were significantly more reduced on

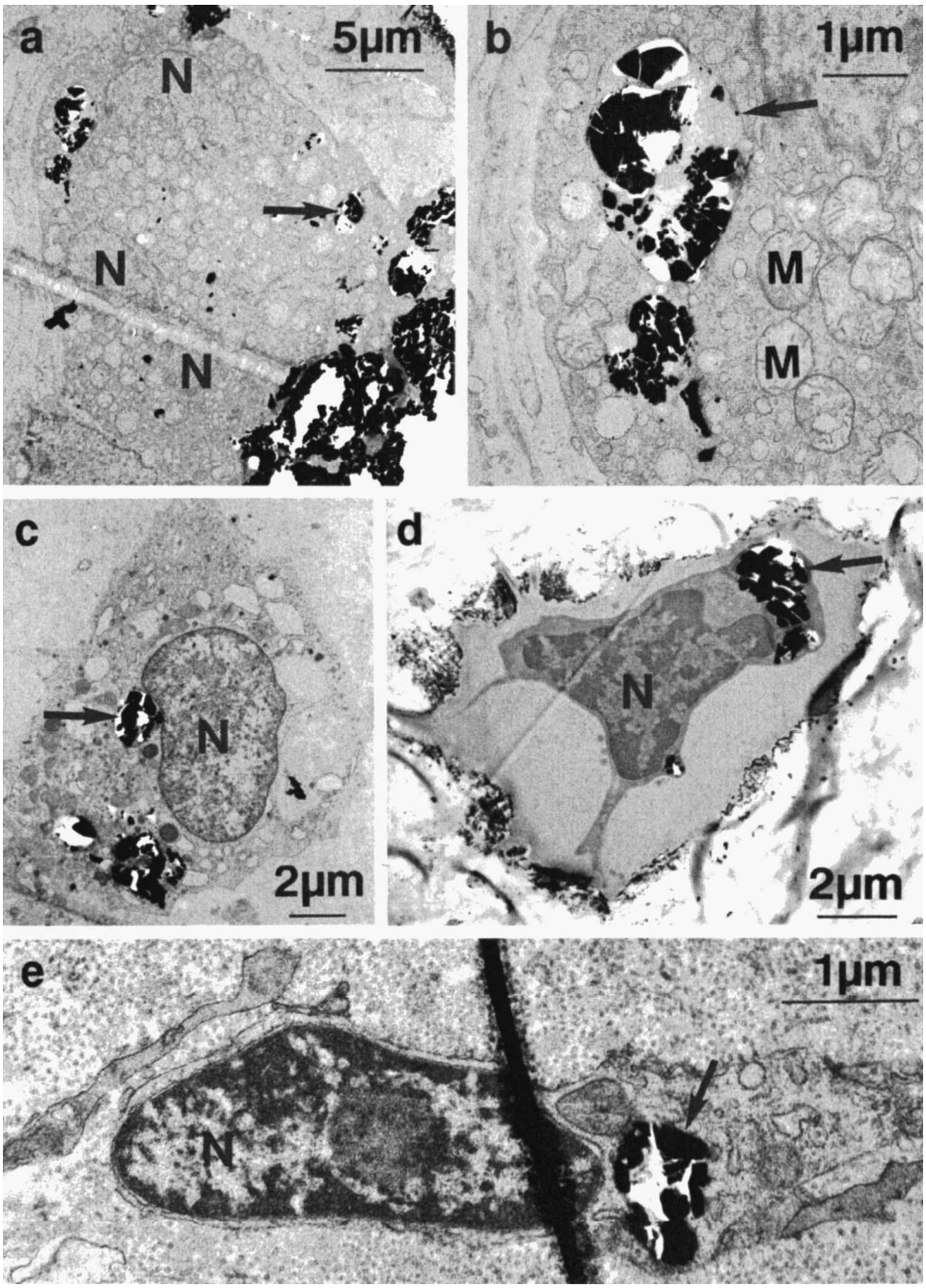


Figure 23. **Transmission electron microscopic images demonstrating apatite crystals in specimens from hydroxyapatite- and fluorapatite-coated implants after 25 weeks implantation.** N=nuclei, M=mitochondria. Arrows indicate crystal containing vacuoles. Sections stained with uranyl acetate and lead citrate. Bars indicate magnification.

a. Multinucleated osteoclast-like cell adjacent to the Ca-P coating. The cytoplasm contains several vacuoles with crystal fragments.

b. Higher magnification of two vacuoles from 4a with granular and apatite crystal content.

c. Mononuclear cell with several apatite containing vacuoles.

d. Osteocyte lying in a lacuna surrounded by mineralized bone matrix. Apatite crystals are shown in the cytoplasm.

e. Fibroblast surrounded by cross cut collagen fibrils. A vacuole containing apatite crystals is shown in the cytoplasm.

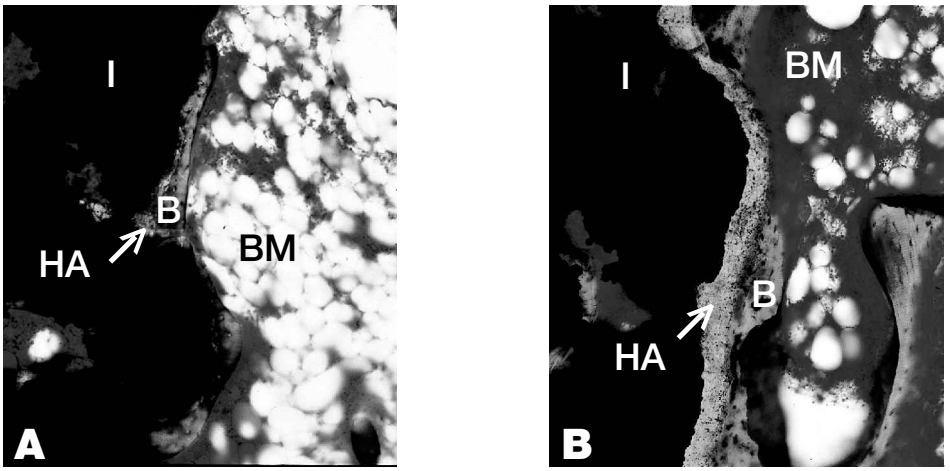


Figure 24. **Histological sections from hydroxyapatite-(HA) coated implants after 16 weeks (Study VII).** A) Implant with 50% crystalline coating (HA-50%) and B) implant with 75% crystalline coating (HA-75%). Both implants have bone ingrowth. Coverage and thickness of the HA coating (arrow) on HA-50% implant is considerably reduced compared to the HA-75% implant. B = Bone, BM = bone marrow. Vertical sections, light microscopy, sections stained with light green and basic fuchsin.

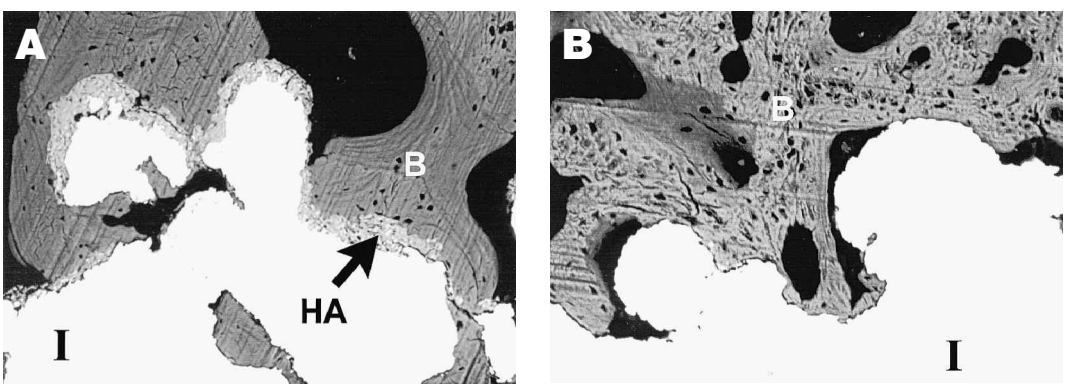


Figure 25. **Backscattered scanning electron images from A) an immobilized implant (I) and from B) a continuously loaded implant (I) (Study I).** The hydroxyapatite (HA) coating (arrow) is reduced significantly on the continuously loaded implant as compared with the immobilized implant. Resorbed HA coating on top of the porous coating on the continuously loaded implant is partly replaced by bone (B) in direct contact with the titanium implant surface. Most of the HA coating on the immobilized implant is covered with bone. Note: no signs of delamination of HA coating on the immobilized implant.

HA-50% implants than on HA-75% implants after both 16 and 32 weeks demonstrating increased resorption of the low crystalline coating (Figure 24). Interestingly, further coating loss was shown from 16 to 32 weeks indicating two phases of resorption (see discussion) (Table 9).

The mechanical factor. The effects of micromotion and immobilization of the implants on coating loss either by a surgical procedure or by bone ingrowth were investigated in study I and III. In study I, HA coverage, absolute surface area and volume were significantly reduced on immobilized implants and further reduced on continuously loaded implants as compared with control implants (Table 9) (Figure 25). Continuously loaded implants had 3-fold reduction in coating surface area and volume as compared with immobilized implants. In study III, 5 out of 8 implants in each experimental group (porous-coated and grit-blasted implants) were stabilized by bone ingrowth whereas the remaining implants were surrounded by fibrous tissue and were found to be unstable.

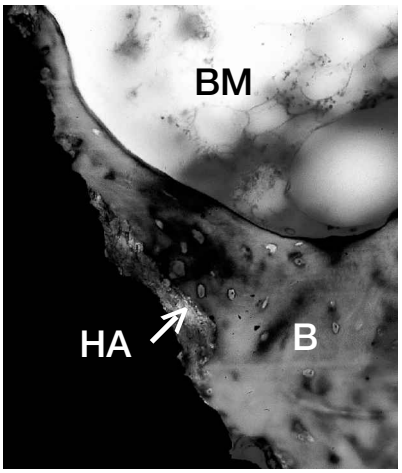


Figure 26. **Histological section from implant coated with a 50% crystalline hydroxyapatite (HA) coating after 32 weeks (study VII).** Resorbed HA coating was partly replaced by bone. Note, bone is in direct contact with the implant surface. B = Bone, arrow = HA, Bar = 200 μm. Vertical section, light microscopy, section stained with light green and basic fuchsin.

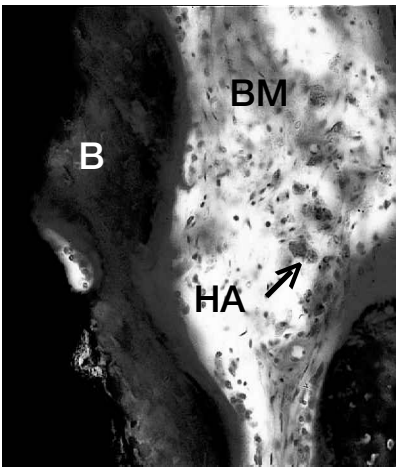


Figure 27. **Histological section from implant coated with a 50% crystalline hydroxyapatite (HA) coating after 16 weeks (study VII).** The HA coating is partially disintegrated and several particles (arrows) have migrated into the bone marrow (BM). Note, bone (B) ingrowth is present and no foreign body reaction is demonstrated. Vertical section, light microscopy, sections stained with light green and basic fuchsin.

Fibrous anchored implants had increased coating loss compared with bony anchored (Table 9) (Figure 19). Thus, micromotion seems to accelerate resorption of HA coatings.

Coating loss replaced by bone. In all studies, completely resorbed coating was partly replaced by bone in direct contact with the implant surface (Figure 25b and 26). Quantitative estimation was not possible in studies I and VII because the implants were incompletely covered with HA before implantation. In study III, 12% of resorbed HA was replaced by bone on porous-coated implants compared with 0% on grit-blasted implants (p=0.03). Study V showed that 36% of resorbed HA coating was replaced by bone ingrowth compared with 29% for FA coatings (p=0.41). In study VII, only 7 HA-coated implants had areas with complete resorption of the coating which were covered by bone marrow or bone tissue. No FA-coated implants had completely resorbed coating.

Delamination and release of particles from the coating

Delamination of the coatings occurred due to cutting and grinding processes. Delamination might also occur in vivo. However, we did not demonstrate any coating delamination which was surrounded by tissue. In all studies (I–VII) HA particles were found incorporated in the bone. When bone marrow was present on the coating surface, particles were also present in the bone marrow but without foreign-body reaction or osteolysis at the LM level. In study VII the HA coating on five HA-50% implants was partially disintegrated after 16 weeks, however, this was not shown for HA-75% coatings (Figure 27). After 32 weeks, most of the disintegrated HA-50% coating was resorbed. The impression was that after 16 weeks more particles were present around HA-50% implants than after 32 weeks, whereas the opposite was shown for HA-75% implants, however, to a minor extent (Not quantified). This might suggest that higher coating crystallinity protects against particle release and coating fragmentation.

Discussion

Bone ingrowth and mechanical fixation

This section will discuss the effects of surface texture, mechanical stability, and Ca-P coating quality on bone ingrowth and mechanical fixation of non-cemented implants.

Significance of surface texture and mechanical stability

Basically, the nature of the implant surface is very important for the events taking place in bone implant interface [326,342,354,387]. However, limited knowledge exists on the reactions within a few microns from the implant surface. It seems that the initial adsorption of proteins is significantly influenced by the topography and chemical composition of the implant surface. At the tissue level, surface roughness of the implant plays an important role for orientation of fibrous tissue to the titanium surface, for bone ongrowth, and for tissue integration to an HA coating [117,239,333,385] (II). During the first weeks after implantation implants with a smooth surface will be encapsulated by fibrous tissue, whereas collagenous fibers will radiate from the rough grit-blasted titanium surface. Goldberg et al showed that later in the repair process, titanium alloy implants with grit-blasted surface had greater bone ongrowth than porous-coated after 6 weeks; however, no difference in mechanical fixation was shown [138]. Along with that we showed that HA-coated grit-blasted implants had higher bone in/ongrowth than porous-coated implants, but without better mechanical fixation (II). In contrast, energy absorption was higher and shear stiffness was lower for porous-coated suggesting that mechanical fixation of porous-coated implants was stronger than grit-blasted implants (II, III). To our knowledge, this is the first controlled study comparing the effects of porous-coated versus grit-blasted surface texture of HA-coated implants. Increased energy absorption was not explained by greater bone ingrowth for porous-coated implants, neither in percentage nor in absolute values. However, it might be explained by the interdigitated bone-implant interface, resulting in a variety of compression, tensile, and shear stresses at the porous surface during push-out testing [124,187] (Figure 28). By contrast, shear stresses overshadow other stresses at the grit-blasted surface.

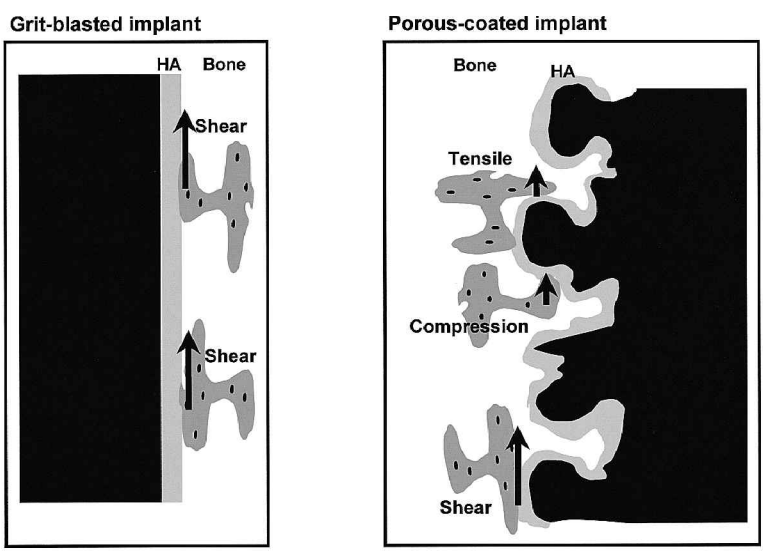


Figure 28. **Stresses at the implant interface during push-out testing of grit-blasted and porous-coated implants.** The interdigitated bone-implant interface of the porous-coated implant results in a variety of compression, tensile, and shear stresses during testing. By contrast, shear stresses overshadow other stresses at the grit-blasted interface.

The interface is also influenced by weight-loading and by the mechanical stability [29,203,367,372,375] (III, VII). Weight-bearing might be beneficial for implant anchorage. Søballe et al. showed that weight-bearing implants were four-fold better anchored than non weight-loaded implants [367]. This might be explained by Wolff's law, which states that bone structure will adapt to the function by orientation of bone trabeculae [420]. However, if the implant is overloaded micromotion might occur. The amount of micromotion is critical to bone ingrowth. Jasty et al. demonstrated that implants subjected to micromotion of 20 μm had bone ingrowth, whereas micromotion of 40 μm resulted in formation of bone but also fibrocartilage and fibrous tissue [203]. In contrast, implants subjected to 150 μm of motion were surrounded by fibrous tissue. This was in agreement with the studies by Søballe et al. who demonstrated that micromotion of 150 and 500 μm resulted in fibrous encapsulation of porous-coated implants [372,375]. By contrast, HA-coated implants had collagenous fibers radiating from the surface after 4 weeks. Furthermore, after 16 weeks the fibrous tissue membrane around HA-coated implants was replaced by bone, whereas non HA-coated implants had fibrous anchorage. In study III, only 10 out of 16 HA-coated implants had bony anchorage when subjected to micromotion of 500 μm during 16 weeks. In addition, four HA-coated implants subjected to micromotion of 250 μm had fibrous anchorage after 16 weeks, whereas all implants were fixed by bone ingrowth at 32 weeks (VII). Thus it seems that micromotion of 500 μm is the threshold for allowing bone ingrowth to HA-coated implants whereas uncoated titanium implants will be anchored by fibrous tissue in the presence 150 μm motion.

Delamination of HA coating. The mechanical fixation of a Ca-P coated implant is influenced by bonding strength between the coating and the metal surface of the implant. Theoretically, the failure site during push-out testing is influenced by several factors including surface texture and bonding between implant-HA and HA-bone and it is a balance between interface stresses and strength [189]. During push-out testing of grit-blasted implants several studies have shown that failure most often occurs within the coating or at the coating-bone interface [134]. However, we found that grit-blasted implants had pronounced delamination of the HA coating during push-out testing indicating higher bonding strength between bone and HA coating than between HA and the metal implant surface (II, III). By contrast, porous-coated implants had no delamination (II, III, IV, VII). This might be explained by poor quality of the HA coatings on grit-blasted coatings. However, the coatings were produced by the same manufacturer by the employment of standard methods as for clinical application and they had identical batch numbers. More likely, delamination on grit-blasted implants might be explained by that higher stresses develop and lower deformation of the interface can occur before failure compared with the interface at porous-coated implant [314]. The HA coating delaminated at considerably lower shear strength than expected from tests preoperatively suggesting that the bonding strength decreased during implantation. This might be explained by the observations made by Clemens et al., who showed that a dry fatigue test did not led to coating failure whereas wet conditions lead to large coating delamination [59]. In addition, resorption of the coating might play a role reducing the bonding strength in vivo.

In our weight-bearing studies none of the Ca-P coatings delaminated in vivo (III, IV, V, VII). By contrast, Shen et al. demonstrated that a weight-bearing rod with a grit-blasted surface had delamination of the HA coating [350]. In addition, David et al. showed that the HA coating with a thickness of 200 μm on a grit-blasted surface was intact after 2 months, however delamination was demonstrated on unstable implants after 9 months indicating that fatigue failure of thick coatings is high[79]. Other studies—again on grit-blasted implants—have shown delamination of the coating in vivo, however without interfering with bone ingrowth [60].

Significance of calcium phosphate coating quality

Hydroxyapatite versus fluorapatite coating. FA was introduced as a more stable coating than HA [97,234]. Moreover, the fact that FA was more thermostable than HA during processing indicated that FA coating was a good alternative to HA [255]. During the last decade several comparative stud-

Table 10. Mechanical and histological results of studies comparing hydroxyapatite-(HA) and fluorapatite-(FA) coated implants

Experimental model	Implant surface	Period (weeks)	Mechanical test	Strength (MPa) HA	Strength (MPa) FA	Bone in/on-growth (%) HA	Bone in/on-growth (%) FA	Author
Goat, press fit, Cortical bone, non-loaded	grit-blasted	12	push-out	13	15 [b]	59	70 [b]	Dhert et al. [96,97]
		25	push-out	17	17 [b]	60	76 [a]	
Goat, press fit, Cortical bone, non-loaded	grit-blasted	12	push-out	14	15 [b]	62	77 [a]	Klein et al. [234]
			push-out	13 [c]	15 [b, d]	73*	77 [b, d]	
Dog, dental screw Mandible, non-loaded	grit-blasted	104	no test			75	77 [b]	Denissen et al. [93]
Goat, press fit Trabecular bone, non-loaded	grit-blasted	12	no test			78	78 [b]	Caulier et al. [49]
Goat, press fit Cortical bone, non-loaded	grit-blasted	6	pull-off	0	0	82	47 [c]	Kangasniemi et al. [212]
		12	pull-off	2.8	0.4 [a]	82	47 [c]	
		24	pull-off	1.2	0.9 [b]	76	61 [c]	
	polished coat.	6	pull-off	0	0	76	0 [c]	
		12	pull-off	4.0	0.7 [a]	91	52 [c]	
		24	pull-off	1.9	0.1 [b]	61	40 [c]	
Goat, gap (1 mm) Trabecular bone, non-loaded	grit-blasted	6	no test			34	21 [b]	Clemens et al. [58]
Dog, gap (0.75mm) Trabecular bone, weight-loaded	porous-coated	25	push-out	6.3	6.1 [b]	60	60 [b]	Overgaard et al. (IV)
Human, gap (1 mm) Trabecular bone, non-loaded	grit-blasted	60	no test			79	61 [a]	Overgaard et al. (VI)
Goat, dental screw Maxilla, non-loaded	grit-blasted	12	no test			36	27	Caulier et al. [50]
		24	no test			35	26	

[a] Significant difference between HA- and FA-coated implants
[b] No significant difference
[c] No statistical analysis
[d] Heat-treated coatings

ies on HA and FA-coated implants have been published (Table 10). It has been consistently shown that FA has the same characteristics as HA with respect to biocompatibility [49,50,58,97,234] (IV, VI). Moreover, we demonstrated that human bone was able to bridge gaps of 1 mm to both HA- and FA-coated implants (VI).

The first studies on FA showed that FA-coated implants had higher bone ingrowth compared with HA-coated implants [97,234]. This was in contrast to our results where no differences between bone ingrowth and mechanical fixation were shown (IV). However, the implants had different surface textures (grit-blasted in contrast to porous coating in our study). Moreover, different coating vendors and animal models might also explain the different results [76]. We used a weight-loaded model in trabecular bone compared to a non weight-loaded transcortical model. Weight-loading might be of special importance as it inhibits bone resorption and initiates bone formation [140,182]. Regarding mechanical fixation, Dehrt et al. showed that HA and FA-coated implants had an ultimate shear strength of 17 MPa for both implant types after 25 weeks whereas our implants reached approximately 7 MPa. This underscores the importance of exercising caution when comparing push-out results from different studies [19,96] (Table 5).

Several studies have later found equal bone ingrowth to HA- and FA-coated implants (Table 10). However, we demonstrated, that HA-coated implants had greater bone ongrowth than FA-coated in humans, however not in dogs, although identical coating qualitites were included (IV, VI).This might be explained by events occurring in the microenvironment early postoperatively in humans where HA presumably releases more calcium and phosphate ions than FA into the bone-

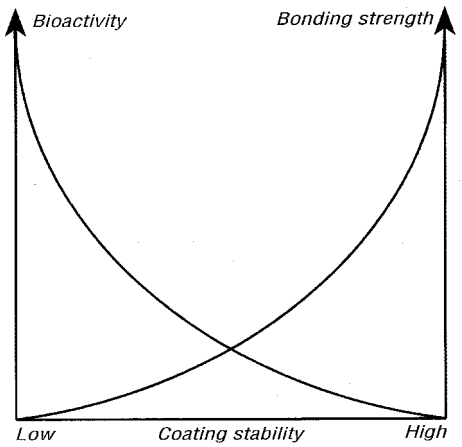


Figure 29. **Relationship between calcium phosphate coating bioactivity and bonding strength to the metal implant surface.** It is hypothesized that low coating stability will result in high bioactivity but low bonding strength and vice versa.

implant interface creating more favourable conditions for bone ongrowth [74].

Only one study has shown greater tensile strength of HA- compared with FA-coated implants [212]. However, the FA coating failed at the coating-implant interface whereas HA tended to fail at the coating-bone interface suggesting a weak bonding of FA to the metal substrate.

Hydroxyapatite coating crystallinity. Generally, for clinical use, none or slow resorption resorbable coatings with high crystallinity have been recommended in order to retain the bonding strength of the coating-implant interface [388]. However, this contradicts the statement that the ideal interface between the implant material and surrounding tissue should match the tissue being replaced. Moreover, HA coating crystallinity has been stated to be one of the most important factors for bioactivity of the HA coating [85]. From this point of view the coating should be of low crystallinity with content of carbonate. However, this might weaken the bonding strength between coating and substrate in vivo (Figure 29). Since one of the first steps in bonding to the coating involves dissolution of the coating surface, it might be suggested that less crystalline or more resorbable coatings would be more beneficial for early bone ingrowth than high crystalline coatings [84,265].

A summary of studies on the effect of coating crystallinity on bone ingrowth and mechanical fixation is presented in Table 11. We showed that implants coated with low crystalline HA yielded better anchorage after 16 weeks than implants with high crystalline HA coating. This was in accordance with Maxian et al. who reported greater strength of grit-blasted implants coated with low crystalline HA after 4 weeks in a non weight-bearing transcortical model [265,267]. The effect diminished after 12 weeks. No effect was shown on implants with a rough surface. These observations might indicate that the effect of crystallinity is in the early postoperative period, an observation which also was evident in our study (VII). In this study, the low crystalline coating did not achieve better anchorage from 16 to 32 weeks whereas the high crystalline coating was stronger fixed after 32 weeks than after 16 weeks. Other studies have shown no effect of low crystalline coating. Nagano et al. reported on a solution precipitated coating on a polyethersulphone implant and demonstrated better fixation of high crystalline coating after 16 weeks, whereas no difference was found after 8 weeks in a non weight-bearing model [274]. Clemens et al. showed no difference in bone ingrowth and gap healing between 30% and 60% crystalline coatings [58] (Table 11). This suggests that the level of coating crystallinity might be critical to bioactivity. Moreover, it might be speculated that different coating crystallinities should be used for different situations to gain increased bioactivity. Thus different coating crystallinity should be used for a stable and an unstable situation. The inconsistent results might also be explained by the fact that assessment of coating crystallinity is conducted by several different methods resulting in different figures for one coating [423].

Our results confirmed the hypothesis by de Bruijn et al. that rapidly resorbable coatings might be more bioactive than slowly resorbable HA coatings [84]. Higher bioactivity of the low crystalline coating might be explained by the physicochemical nature of the coating in the local microenvironment. A low crystalline coating releases more calcium and phosphate ions than the high crystalline coating due to dissolution and cell mediated resorption enhancing bone formation and

Table 11. Mechanical and histological results of studies comparing hydroxyapatite (HA) coating crystallinity

Experimental model	Implant surface	Period (weeks)	Crystallinity	Shear strength (MPa)		Bone in/on-growth (%)		Author
Rabbit, interference fit,	grit-blasted	4	low vs. amorphous	2.5	1.9 [a]	85.6	82.4	[c] Maxian et al. [265]
cortical bone, non-loaded	grit-blasted	12	low vs. amorphous	3.5	3.4 [b]	82.3	83.2	
	rough	4	low vs. amorphous	3.5	3.0 [b]	76.0	83.5	
	rough	12	low vs. amorphous	6.2	6.4 [b]	77.0	81.3	
Rabbit, interference fit,	rough	2	low vs. amorphous	2.7	2.0 [b]	not reported		[c] Maxian et al. [267]
cortical bone, non-loaded	rough	4	low vs. amorphous	1.9	2.2 [b]	64.6	79.8	
	rough	12	low vs. amorphous	2.9	3.2 [b]	73.3	80.6	
Rat, press fit, cortical bone, non-loaded	grit-blasted	1,2,4	10%, 60%, 95%	not performed		qualitative [d]		de Bruijn et al.[84]
Goat, press fit, cortical bone, non-loaded	grit-blasted	12	low vs high [e]	12	14 [b]	61.8	73.3 [b]	Klein et al. [234]
Dog, gap,	porous-coat.	16	50% vs. 75%	7.2	3.4 [b]	29	16 [a]	Overgaard et al.
trabecular bone,		32	50% vs. 75%	8.4	11.7 [b]	34	40	(VII)
weight-loaded, micromotion								
Goat, gap, 1 mm gap	grit-blasted	6	25-30% vs. 60-63%	not performed		27	34 [b]	
trabecular 2 mm gap	grit-blasted	6	25-30% vs. 60-63%	not performed		16	10 [b]	
bone, non-loaded								Clemens et al. [58]

[a] Significant difference
[b] No significant difference
[c] Results of bone ongrowth are based on two implants in each group
[d] 10% HA elicit earlier bone formation than 95%crystallinity
[e] heat-treated coating,

bonding [85,151,243,266]. At the ultrastructural level, de Bruijn et al. showed high surface reactivity on the low crystalline coating indicated by collagen fibres perpendicularly aligned to the surface and by the incorporation of afibrillar globules in the degrading surface [85]. In addition, greater surface activity of low crystalline HA coating was shown to enhance cell attachment and spreading as compared with high crystalline coatings which is in accordance with a recent published in vitro study [55].

Whether or not mechanical fixation of implants with low crystalline coatings will diminish in the long run can be questioned; at present, no data supports this hypothesis for plasma-sprayed HA coatings. On the other hand, reports on TCP coatings in vivo have shown inferior fixation strength as compared with HA, most likely due to rapid coating resorption [228,245]. It can be speculated that the bioactivity of an HA coating will reach a maximal level at a certain crystallinity and that there is a balance between bioactivity and interface strength (Figure 29).

Comparison of HA coatings produced by different methods

Few studies have compared the biological effects of Ca-P coatings produced by different methods. Lacefield investigated the mechanical properties and adherence of 4 different coatings: hot isostatic pressing, sputter coating, dip/sintering coating, and immersion coatings [238]. He concluded that sputter coating appeared to be the method of choice for a dense adherent HA coating. No difference between magneton-sputter and plasma-sprayed HA coatings and between plasma-sprayed and a high velocity flame-sprayed HA coating were found [190,286]. Wang et al. compared a sintered high crystalline HA coating with a plasma-sprayed low crystalline coating and found more resorption of plasma-sprayed coatings but no difference in mechanical fixation [405]. In conclusion, it seems that coating quality rather than coating method is of importance for the biological performance of a coating in vivo.

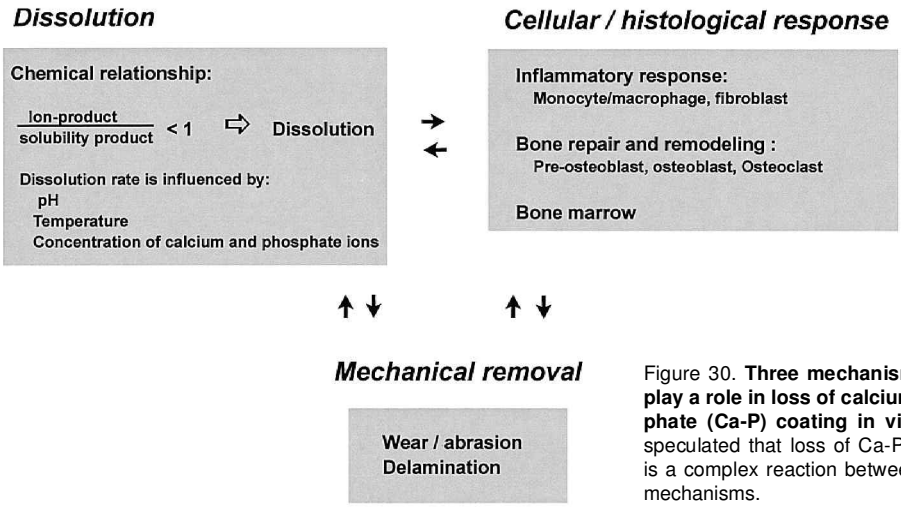


Figure 30. **Three mechanisms may play a role in loss of calcium-phosphate (Ca-P) coating in vivo.** It is speculated that loss of Ca-P coating is a complex reaction between these mechanisms.

Loss of calcium phosphate coating in vivo

The clinical use of HA coating remains a controversial issue especially due to concerns regarding the long-term performance of the coating and the effects of resorption. Resorption has been claimed to influence implant stability and to cause delamination of the coating resulting in third body wear debris, and ultimately failure of the implant [9,21,22,43,101,200,271]. Several mechanisms might contribute to coating loss in vivo and numerous factors might influence the process.

Mechanisms of coating loss

Three mechanisms may play a role in loss of HA coating in vivo; simple dissolution, cell-mediated resorption, and mechanical removal. However solid documentation at the in vivo level does not exist in the literature [8,9,201,305] (Figure 30).

Simple dissolution. In the body environment, a Ca-P coating will dissolve under restricted conditions due to dissolution kinetics determined by ionic and solubility product [57]. Local undersaturation of the fluid with calcium and phosphate ions as determined by the thermodynamic solubility product leads to coating dissolution. It might be expected that different qualities of Ca-P coatings have the same properties in the same physiological environment due to saturation with calcium and phosphate in the extra-cellular fluid. However, several studies have shown varying degradation behaviour demonstrating that the thermodynamic surface equilibrium is not necessarily reached in vivo which might in turn be explained by fluctuation in Ca-P concentrations [225,283]. Dissolution in vivo is probably a mandatory process in triggering bone formation on Ca-P coatings and the process has been compared to that of dissolution of endogenous bone mineral [76,82,115,230,233,266,394]. In vitro, HA dissolution increases with decreasing pH in various buffer solvents. In addition, dissolution of HA is enhanced by increased impurity, low crystallinity, small neck size, and high porosity [76,83,227,266,267,427]. Some coatings also appear to dissolve at neutral pH, in particular, those with low crystallinity and high impurities. Solubility of FA is lower than HA especially at low pH, and it is increased by low crystal size of both HA and FA, whereas solubility of TCP is higher than that of both FA and HA [81,100,105,230,233,319]. Crystal size of FA is greater than HA which in combination with the crystal lattice explains lower solubility of FA compared to HA (Figure 1). Heat-treatment decreases solubility of HA coatings whereas no changes occur for FA coatings.

Cell-mediated resorption. Cell-mediated bone resorption is caused by several cell types. However the osteoclast is the only cell specialized for that function [6]. Monocytes and macrophages are also capable of degrading both inorganic and organic bone matrix [102,152]. They release local factors contributing to resorption of bone but without formation of lacunae specific to the osteoclast.

Cell-mediated resorption of Ca-P coatings has been documented in several studies. In vitro, the osteoclast can create resorption lacunae due to low pH at the ruffle border [82,83,115,394]. De Bruijn et al. showed that osteoclastic resorption lacunae were present only when the coatings were pre-cultured with primary osteoblasts indicating that the osteoclasts were activated by the conditioned media [83]. They speculated that extracellular matrix proteins may play a role in osteoclast stimulation. The role of early postoperative osteoclastic resorption of HA is most likely minor due to few osteoclasts at that stage; however, later, when bone ingrowth has occurred, osteoclastic resorption might take place. We demonstrated the presence of HA and FA particles arranged in osteoclast-like cells, monocytes, and fibroblasts in agreement with earlier reports [82,141,147,273,288,294,397]. Moreover, we found crystal fragments inside osteocytes. The significance of this is not clear, but the particles might have been incorporated in the pre-osteoblast or osteoblast stage [273]. Phagocytosed HA fragments might undergo phagosomal solubilization followed by release of calcium and phosphorous ions into the cytoplasm [114,147]. Cell-mediated resorption seems to be influenced by surface topography. It was demonstrated that the number of osteoclast-like cells from rat bone marrow was greater on rough than on smooth surfaces [82,141]. Whether this finding is true for the in vivo processes and for other cell types remains uncertain. However, studies II and III showed that HA coverage was decreased on porous-coated compared with grit-blasted implants.

Whether cell-mediated coating resorption is caused by solution mediated dissolution, due to local cell activity, or due to phagocytosis of released coating fragments, is unknown. This makes it difficult to distinguish between dissolution and cell-mediated resorption.

Mechanical removal. Mechanical removal by wear or abrasion and delamination might play a role in coating loss. Detection of abrasion or wear particle requires element analyses at the EM level. Most likely, particles are released from the HA surface. Study VII demonstrated that numerous particles were present around implants with low crystalline HA coating, indicating that the magnitude and rate of particle release are influenced by coating quality. For higher crystalline coatings, only few particles were present (I, II, III, V, VI, VII).

It is also hypothesized that particle release is accelerated by micromotion. Release of HA particles from a low crystalline coating might be due to heterogeneity in coating crystallinity [150,152]. Whether HA particles are critical for the interface in regard to bone ingrowth is a matter of debate. Several in vitro studies have demonstrated increased cytokine production from macrophages, osteoblasts and osteoclasts when exposed to HA particles smaller than 50 μm [164,181,281,364,365]. In vivo, Goodman et al. reported that HA particles did not elicit a chronic inflammatoric response or abnormal bone remodeling when implanted into the medullary cavity of rabbits [143]. Whether the number, shape and size of the HA particles used in vitro are relevant to the clinical situation is unknown, as no study on HA particles retrieved from clinical case has been published. Moreover, the fact that HA particles in several clinical series often are surrounded by bone or bone marrow without a foreign reaction in vivo coupled with absence of radiolucent lines questions the clinical significance of current in vitro studies [44,72,136].

Whether dissolution, cell mediated resorption or mechanical removal is the most active mechanism for coating loss in vivo is unknown. The specific conditions in the microenvironment will determine the reaction which most likely is an interaction between the mechanisms and, in the case of stable condition an interaction between dissolution and cell-mediated resorption.

Factors affecting coating loss

Several factors might affect loss of Ca-P coatings in vivo (Figure 31). They can be categorized as coating related, mechanical, biological, and as implant related factors. The factors interact with each other and will act through one or more of the three mechanisms of coating loss described above (Figure 30). This discussion part will focus on factors which affect loss of Ca-P coatings in vivo.

Coating related factors

The coating type, application method and coating quality are of great importance for loss of Ca-P coatings in vivo.

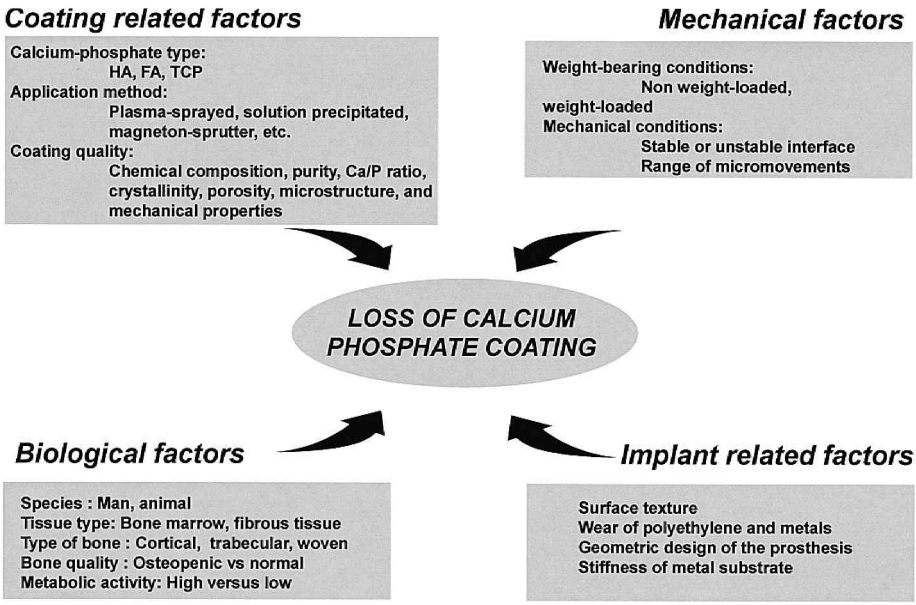


Figure 31. **Several factors related to 1) the coating, 2) mechanical condition, 3) biology, and 4) the implant and might affect loss of calcium phosphate coatings in vivo.** Each factor will act through one or more of the pathways as listed in figure 30.

Calcium phosphate type. We investigated loss of HA and FA coatings in dogs and humans demonstrated significant resorption of both coatings with no difference in overall resorption rates (V, VI). By contrast, Klein et al found that heat-treated FA coatings were more stable compared with corresponding HA coatings [233]. Dhert et al. and Clemens et al. employed HA and FA coatings from the same vendor and found similar results [58,97]. This might be explained by variations in experimental and different coating vendors, resulting in coatings of different qualities as well as by different assessment methods [76,92]. An interesting finding in our study was that only modest resorption of the coatings was found when bone was present at the coating surface and resorption was lower for HA than for FA (VI). This might be explained by faster bone ongrowth to HA-coated implants, postoperatively. By contrast, when bone marrow was present at the coating surface, HA was resorbed more than the FA coating, most likely due to greater solubility of HA, particularly at low pH levels [233,289]. Despite the presence of fibrous tissue around parts of FA-coated implants in humans, the coating was still preserved in accordance with the findings of Caulier et al. [50].

Studies comparing HA and TCP coatings have repeatedly shown that TCP is resorbed much faster than HA coatings [225-227,231,245,325]. The different resorption rates of HA, FA, and TCP are explained by the crystal structures and differences in dissolution kinetics.

Coating quality. Coating quality is important to bioactivity and the rate of coating loss. Moreover, several parameters for coating quality are important for coating loss (Figure 31). Several studies have demonstrated that the lower the Ca/P ratio, the more rapidly the coating will be resorbed [225,366]. In addition, it has been stated that porosity and microstructure are important for resorption [83,227,229,258]. Coating crystallinity has been investigated extensively due to its significant impact on bioactivity and resorption of pure HA coating.

Crystallinity. Coating crystallinity is very important for resorption of Ca-P's. During unstable and weight-bearing conditions, we demonstrated increased loss of low crystalline HA coating with regard to coverage and coating thickness after both 16 and 32 weeks (VII). This is explained by greater solubility of the low crystalline coating due to dissolution kinetics as described above.

Moreover, low pH will increase dissolution of the low crystalline coating [233,267,310]. In addition, cell-mediated degradation and most likely mechanical removal played a role, as well. These results are in agreement with earlier studies which, however, were conducted in non-weight-bearing models and often with a heat treated coating as the high crystalline coating [49,58,93,234].

Interestingly, no further coating loss was observed from 16 to 32 weeks, suggesting two phases of coating resorption (VII): *Phase I* with rapid coating loss, and *phase II* with slow loss. The phases might be explained by the metabolic activities postoperatively and by heterogeneity in the coating (see below).

Biological and mechanical factors

The significance of micromovements on coating loss was investigated in study I. Significant loss of the HA coating on both immobilized and continuously loaded implants was demonstrated. Continuous loading and micromotion of the implant accelerated resorption. In addition, study III showed that coating loss was enhanced on unstable fibrous anchored as compared with bony anchored implants. The difference in coating loss between stabilized implants and implants with continuous micromotion is most likely explained by the biological reactions: Two phases of coating loss is suggested to occur. *Phase I* with rapid coating loss, and *phase II* with slow loss. During *phase I* the implant is subjected to micromotion and a fibrous tissue membrane with high metabolic activities is developed [38]. The fibrous tissue membrane is dominated by fibroblasts and macrophages able to phagocytose HA [141,294,375,397,(V)]. Because of unstable conditions, low pH is maintained due to inhibited angiogenesis. In addition, fluid flow and pressure are increased along the interface leading to accelerated dissolution due to changes in calcium and phosphate concentrations [308,320]. During *phase I,* the low crystalline coating parts will be resorbed leaving the more crystalline coating on the implant surface. In addition, coating crystallinity might eventually increase with time thus possibly contributing to low coating loss in *phase II* [149]. *Phase II*, begins by stabilization of the implant when the initially formed fibrous tissue membrane is transformed to bone through endochondral ossification. This occurs at least 8 weeks postoperatively, as suggested by fluorochrome labelling (III, VII) [372]. If the implant is not stabilized, phase *I* will continue and complete coating loss might occur.

Tissue type was also demonstrated to be important for coating loss. The presence of bone marrow and fibrous tissue on the coating surface increased resorption compared with bone (V, VI). Several studies have confirmed reduced loss of HA and FA coatings when bone ingrowth had occurred compared to ingrowth of bone marrow or fibrous tissue, however, not quantified [10,253,295,311]. Piattelli et al. stated that resorption of HA coating when covered by bone marrow was caused by mono- and multinuclear cells which stained positive for acid phosphatase [311].

Differences in weight-loading conditions were not investigated, however our studies suggest that weight-loading conditions may play a role in loss of HA (Table 9). Moreover, it might be speculated that resorption during non-loading conditions is decreased in humans compared to dogs because of lower metabolic activities.

Implant related factors. The design of a prosthetic component might influence coating loss due to micromotion or stresses at particular parts of the prosthesis. Other factors such as wear debris, might also contribute to accelerated coating loss due to third body wear processes. In addition, surface texture is critical to the interface reactions. This might be explained that HA coverage was more reduced on porous-coated as compared with grit-blasted implants (II, III). In addition, the fluid flow and pressure along the unstable implant interface might create different microenvironments at the interface to porous-coated and grit-blasted implants [320]. Stiffness of the prosthetic component might also affect coating loss increasing the risk of coating delamination.

Significance of HA coating loss. Is it preferable that the HA coating retains on the substrate surface or should the HA coating be resorbed in the long-term run? Ducheyne pointed out that it cannot be reasonably expected that the mechanical function of the HA coating can last for the patient's lifetime and suggested that a coating must necessarily be resorbed [101]. Coating loss in vivo might be critical first of all for bone ingrowth

and secondly for implant fixation. How rapid the HA coating can be resorbed without disturbing bone ingrowth to the implant surface is a balance between release of calcium and phosphate ions and bone formation. In study VII, it seems that the balance for both low and high crystalline HA coating favoured bone ingrowth. However, the rate of resorption is most likely important for implant fixation, since implants coated with rapidly resorbed coatings like TCP have been found to be inferiorly anchored compared to a slowly resorbed HA coating when applied to a grit-blasted surface [231,245]. This addresses the importance of underlying surface texture. If the coating is completely resorbed, implant fixation is solely provided by the metal surface. In that case, a rough or porous-coated surface will probably provide stronger implant fixation than a grit-blasted surface.

Whether HA coating loss can reduce implant fixation when bony anchorage has occurred is doubtful. In the present studies, coating loss did not seem to interfere with bone ingrowth and resorbed coating was partly replaced by bone which suggests firm implant fixation (I–VII). Coating loss due to extensive delamination might be a severe problem of coating quality and might result in implant loosening. This was, however, not shown in our studies.

Clinical implication of hydroxyapatite coatings

The clinical use of HA-coated prostheses is well-established. Thus HA coating is capable of reducing the early migration of both femoral hip and tibial knee components as compared with uncoated implants when detected by roentgen stereophotogrammetric analysis [215,218,275,291,292,329,380]. In addition, Geesink et al. reported six-year results of HA-coated total hip replacement and found a survival rate of 100% for the stems and 99% for the threaded cups [136]. HA might also reduce migration of polyethylene particles along the bone-implant interface as demonstrated experimentally [324]. This sealing effect could be very important, reducing extension of osteolytic lesions and eventually failure of the implant.

Whether the firm fixation of HA-coated prosthetic components will last for a longer time period than uncoated or cemented prostheses has yet to be elucidated. According to Kärrholm et al. mechanical loosening can be predicted from roentgen stereophotogrammetric analysis during the first two years [214]. Hence, there is a strong indication that HA-coated prostheses will remain stable to a larger degree than non-HA-coated prostheses, which could result in a superior survival rate. However, whether or not a bony anchored prosthesis will remain stable depends on the bonding strength of the coating to the prosthetic surface and on loss of coating. This addresses the importance of coating quality [378]. With regard to coating loss, retrieval studies have reported evidence of HA coating loss in accordance with our results [10,167,253,391]. If the coating is partly replaced by bone, as our studies suggest, then the implant fixation might be durable.

A few retrieval studies have shown failure of HA-coated prosthetic components [39,278]. Nilsson et al. reported delamination of HA coating from a tibial tray 7 months after insertion [278]. The HA coating was 200 μm thick and the metal implant surface was grit-blasted with a macrotexture. In addition, Buma et al. showed loosening of an HA-coated femoral hip component 2 years after primary operation. Histology demonstrated complete loss of the HA coating from the grit-blasted implant surface. In a series of 94 consecutive HA-coated THA's, Røkkum et al. showed excessive polyethylene wear and loosening of 5 cups within 5 years after surgery [337]. However, the implant was a hemispheric screw cup with a grit-blasted surface texture and no control group was included [303]. The screw cup design has in several studies shown higher revision rates than porous-coated and cemented cups [425]. The significance of HA particles and their contribution to third body wear has been discussed [11,21,23,271]. Bloebaum et al. reported that HA particles were present in PE inserts, but did not include control inserts from patients without HA-coated implants [21]. However, Frayssinet et al. demonstrated Ca-P particles in PE inserts from patients with both HA and non HA-coated prostheses and concluded that Ca-P could crystallize in cracks or crevices of PE inserts [120]. Bauer et al. found that surface roughness on modular heads from HA-coated THA's were signifi-

cantly lower than on heads from cemented and porous coated uncemented implants [11]. They concluded that third body wear was of no more severity in HA-coated implants that in cemented or porous-coated implants. Finally, Morscher et al. reported that third body wear due to HA particles may produce severe osteolysis [271]. However, the HA coating with a thickness of approximately 300 μm was applied on a polyethylene cup which initially was fixed by two pegs and dowels of polyacetal. However, the identical cup but without HA inserted non-cemented has showed excessive polyethylene wear and a failure rate of up to 57% after a mean follow-up of 6.3 years [254,418]. The cup is now abandoned.

In conclusion, the clinical performance of HA-coated prostheses is promising and might increase the survival rate of uncemented prostheses, however, long-term follow-up still remains to be evaluated. It seems that HA coating should be used on a porous-coated surface since failure of the HA coating has been shown on prosthetic components with a grit-blasted surface in accordance with our studies (II, III). HA coating should not be used to improve a poor implant design or to improve the surgical technique.

Conclusion

The present series of studies demonstrated that Ca-P coating type and quality together with the underlying surface texture had significant influence on either mechanical fixation, bone ingrowth or loss of coating in experimental models in dogs and humans. In addition, the significance of systematic sampling in histomorphometry was shown.

Effects of surface texture on mechanical fixation and bone ingrowth: porous-coated versus grit-blasted (II, III)

HA-coated implants with plasma-sprayed porous-coated implant surface exhibited stronger anchorage than implants with a grit-blasted surface when inserted into trabecular bone during non-weight-bearing and weight-bearing conditions with micromovements of 500 μm. Macroscopically, the HA coating delaminated on grit-blasted implants whereas porous-coated implants predominantly failed at the HA-tissue interface.

Effects of calcium phosphate coating quality on mechanical fixation and bone ingrowth (IV, VI, VII)

In dogs, HA- and FA-coated implants showed no differences in bone ingrowth and mechanical fixation. Moreover, no difference in bone remodeling around HA- and FA-coated implants was demonstrated (IV). In humans, HA-coated implants had significantly greater bone ongrowth than FA-coated implants (VI).

Low crystalline (50%) HA coating accelerated early mechanical fixation and bone ingrowth compared with high crystalline coating (75%) during weight-bearing conditions and micromovements of 250 μm (VII). Implants with low crystalline coating did not achieve better anchorage from 16 to 32 weeks. By contrast high crystalline coating gained significantly better anchorage from 16 to 32 weeks.

Factors affecting loss of calcium phosphate coatings in vivo (I–III, V–VII)

In all studies, the Ca-P coatings were significantly reduced irrespective of type and quality. Completely resorbed coating was partly replaced by bone suggesting durable implant fixation. HA coverage on porous-coated implants was significantly reduced compared with grit-blasted implants (II, III). No differences in overall resorption parameters between HA and FA coatings in dogs and humans were demonstrated (V, VI). In humans, significantly less HA and FA coating was resorbed when bone was present on the coating surface compared to presence of bone marrow or fibrous tissue (VI). HA was resorbed more than FA in the presence of bone marrow. Resorption was lower for HA than for FA when bone was present on the coating.

Low crystalline coating was significantly more reduced after both 16 and 32 weeks compared with high crystalline HA coating (VII). No further coating loss was observed from 16 to 32 weeks suggesting two phases of coating resorption: *Phase I* with rapid coating loss, and *phase II* with slow loss.

Continuous loading and micromovements of 150 μm accelerated resorption compared with immobilization of the implant (I). In addition, unstable fibrous anchored implants had significantly more loss of HA coating as compared with bony anchored implants (III).

Efficiency of systematic sampling (VIII)

It was demonstrated that biological variance was the major contributor to the total observed variance in bone histomorphometry using the vertical section method. Optimizing the sampling design could significantly reduce work load at the hard-tissue microtome and the microscope without reducing the quality of data, which remained unbiased due to application of stereological methods. The most efficient way to reduce the total variance of group mean values in biological studies would be to include more individuals.

Suggestions for future studies

Future research should focus on

- Improvement of coating quality and implant fixation in primary and revision surgery,
- Coating resorption in vivo,
- Histomorphometric methods.

Improvement of coating quality and implant fixation. Early implant fixation might be enhanced by more bioactive Ca-P coatings. Dual HA coatings, consisting of a an outer layer of more resorbable HA and an inner layer of a more stable coating might be advantageous in order to enhance implant fixation and to minimize the risk of coating disintegration and delamination. New coating techniques have emerged; these might prove to exceed today's golden standard (the plasma-spraying method). The advantages of magneton-sputter and solution precipitated coating are that they cover the entire porous implant surface and that the they might be very thin (5–10 μm) [190,408,421].

To avoid use of Ca-P coatings surface treatment of the metal implant surface by increased oxidation or protein adsorption appears promising and might be an alternative to HA coatings in addition to new surgical techniques such as in situ bone compaction [51,61,146,162,408]. The revision cavity is completely different from the primary bone bed and Ca-P coatings are poorly investigated with respect to healing capacity and could be further analyzed [13].

Stimulation of early bone ingrowth by combining osteoconductive HA coatings and osteoinductive factors is another area of interest both in primary and revision surgery [204,248,368]. Application of growth factors seems promising, however, the optimum growth factor or combinations of growth factors in addition to dose-related problems have to be investigated. In the revision situation growth factors might be advantageous in combination with bone graft materials such as auto- or allograft or bone substitutes. Gene therapy may be a new important delivery system of growth factors [279]. Gene based delivery systems offer the potential to achieve therapeutic levels of growth factors for longer time periods than exogenously delivered proteins. Their role in bone implant research needs to be investigated.

Coating resorption in vivo. The present studies demonstrated that several factors influenced loss of Ca-P coating in vivo. However, the mechanisms for coating loss in vivo are poorly understood and should be investigated for better understanding of the basic processes. This would require in vivo analyses of the interface chemistry and kinetics and evaluation of the coating before and after implantation. In addition, the effects of HA particles on implant fixation should be evaluated in clinical relevant models.

Histomorphometric methods. In stereology, the effect of deviation from the perfect vertical axis in the vertical section method should be simulated mathematically. In addition, sampling efficiency might be further optimized.

Acknowledgments

This study was financially supported by grants from the Danish Rheumatism Association, Danish Medical Research Council, University of Aarhus, Denmark, VELUX Foundation, Novo-Nordisk Foundation, Karen Elise Jensen Foundation, Ydes Foundation and Danish Medical Association Foundation. Biomet Inc., USA and DK kindly provided the implants, and the Ca-P coatings were kindly provided by BioInterfaces Inc., USA.

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Dansk resumé

De osteoconductive egenskaber (evnen til at lede knogle vækst) hos calcium fosfat coatninger (CFC), hydroxyapatit (HA) og fluorapatit (FA), er veldokumenterede i såvel eksperimentelle som kliniske studier. HA coatede implantater anvendes i klinikken med det formål at accelerere den tidlige protese forankring. Den tidlige protese forankring har vist sig at være en god prædiktor for proteseoverlevelse. HA er i stand til at øge knogle indvæksten til implantater. Desuden er det vist at HA kan nedsætte migrationen af protese komponenter ved hofte- og knæ-proteser. Trods den kliniske anvendelse af HA-coatede proteser, er der flere relevante aspekter omkring HA coatninger, der ikke er afdækket. Den optimale coatnings kvalitet kendes ikke. Desuden er der ikke enighed om, hvilken overfladestruktur det underliggende implantat skal have. Herudover diskuteres betydningen af coatningens resorption. Det har været fremsat at resorption af coatningen vil medføre nedsat bindingsstyrke mellem coatning og metal overflade og mellem implantat og knogle, og at dette kan føre til delaminering af coatningen, dannelse af partikler og ultimativt løsning af implantatet.

Formålet med denne afhandling var at undersøge effekterne af forskellige CFC. Betydningen af coatnings type og kvalitet og af implantatets overfladen blev analyseret med henblik på bestemmelse af mekanisk fiksation og knogle indvækst. Herudover blev en række faktorer med indflydelse på coatningens resorption afdækket. Til histologisk analyse blev anvendt stereologisk metoder for at opnå resultater uden bias. Endelig blev betydningen af systematisk sampling ved anvendelse af den stereologiske vertikale snit teknik ved knogle histomorphometri analyseret.

Resultater. De første studier analyserede effekten af porøs versus sandblæst implantat-overflade begge coatet med HA. Klinisk anvendes proteser med begge overfladetyper. Implantater med porøs overflade var bedre forankret end sandblæste implantater både ved ikke vægtbelastede og ved belastede implantater med tilstedeværelse af mikrobevægelser. Ved mekanisk testning delaminerede HA coatningen på implantater med sandblæst overflade, hvilket ikke blev observeret på porøse overflade. Knogleindvæksten til sandblæste implantater var procentuelt større end til porøse. Dette indikerer at overflade-beskaffenheden har indflydelse på den biologiske aktivitet på implantatoverfladen.

Den næste serie af studier undersøgte effekten af CFC type, HA versus FA. FA er ved tidligere studier fundet at være mere kemisk stabil end HA, desuden har nogle studier indikeret at FA har større bioaktivitet end HA. Vi fandt ingen forskel i mekanisk fiksation og knogle-indvækst til porøst coatede implantater i hunde under vægt-belastede betingelser. Derimod blev der i et human studium fundet større knogleindvækst til HA end til FA coatede implantater et år efter indsættelse.

Det næste studium analyserede effekten af HA coatningens kemiske struktur, krystalliniteten. Coatningens krystallinitet kan være væsentlig for coatningens bioaktivitet. Der blev anvendt en hurtig resorberbar lav-krystallin coatning (HA-50%) versus en høj- krystallin langsommere resorberbar coatning (HA-75%). Efter 16 uger blev der fundet bedre forankring af implantater med HA-50% og desuden en større knogle indvækst end til implantater med HA-75%. Derimod var der ingen forskel efter 32 uger, hvilket indikerer at HA-50% accelererer den tidlige implantat fiksation.

I forbindelse med alle nævnte studier blev resorption af coatningen undersøgt. Alle coatningstyper blev reduceret under implantationsperioden. Dækningsgraden af HA blev væsentlig mere reduceret på porøst coatede implantater i forhold til sandblæste. Der var ingen forskel i den overordnede resorption af HA og FA. Imidlertid viste det humane arbejde at resorptionen af HA var større end FA ved indvækst af knoglemarv og fibrøst væv, mens dette ikke var tilfældet, når der var knogle indvækst. Den lav-krystalline coatning (HA-50%) havde større resorption end HA-75% efter både 16 og 32 uger, men der blev ikke fundet yderligere resorption fra 16 til 32 uger. Dette indi-

kerer to faser ved resorption af HA coatninger: *Fase 1* med et hurtigt tab af coatningen på grund af større metabolisk aktivitet, mikrobevægelser og lavt pH, og en *fase 2* med langsom resorption af coatningen. Der blev yderligere fundet at mikrobevægelser accelererer resorptionen af HA. Ved elektron mikroskopi blev fundet at flere celletyper indeholdt HA fragmenter, hvilket indikerer at det cellulære respons spiller en rolle ved resorption af CFC. I alle studierne blev den resorberede coatning delvist erstattet af knogle indvækst hvilket indikerer en holdbar implantat forankring.

Ved analyse af systematisk sampling med anvendelse af den stereologiske vertikale snit teknik blev fundet, at antallet af snit og synsfelter og at gridens tæthed havde betydning for variansen udtrykt som "coefficient of error". Kun mindre ændringer i variansen blev fundet ved reduktion af tælleindsatsen fra at tælle alt på begge implantatsider på 14 snit til at tælle én side og kun hvert tredje synsfelt med 50 % densitet af tælle-griden. Dette kunne reducere arbejdesindsatsen ved mikroskopet med 90 %. Herudover blev det vist at antallet af snit kunne reduceres til 3-4 snit per implantat uden væsentlig stigning i variansen. Endelig blev det vist at den biologiske variation udgjorde det største bidrag til variansen. Dette betyder at ønskes variansen nedsat gøres dette mest effektivt ved at inkludere flere individer i et studium.

Der konkluderes at typen, kvaliteten af CFC og strukturen af implantat overfladen har væsentlig betydning for enten implantat fiksation, knogleindvækst og/eller resorption af coatningen. Arbejdsindsatsen ved såvel mikrotom som ved mikroskop kunne nedsættes betydeligt ved anvendelse af systematisk sampling med den stereologiske vertikale snit teknik.