The Influence of Local Bisphosphonate Treatment on Implant Fixation

PhD thesis

Thomas Jakobsen

Faculty of Health Sciences
University of Aarhus
Denmark
2008

From
Orthopaedic Research Laboratory
Department of Orthopaedics
Aarhus University Hospital
Aarhus Denmark

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“Science may be described as the art of systematic over-simplification”.
Karl Popper (1902-1994)

List of Papers

This PhD thesis is based on the following papers:

I. Local Alendronate Increases Fixation of Implants Inserted with Bone Compaction. 12-week Canine Study. Jakobsen T, Kold S, Bechtold JE, Elmengaard B, Søballe K.
J.Orthop.Res.; 2007 Apr;25(4):432-41.

II. Soaking Morselized Allograft in Bisphosphonate can Impair Implant Fixation. Jakobsen T, Baas J, Bechtold JE, Elmengaard B, Soballe K.
Clin Orthop Relat Res; 2007 Oct;463:195-201.

III. Topical Alendronate Treatment Increases Fixation of HA-coated Implants Inserted with Bone Compaction. Jakobsen T, Baas J, Elmengaard B, Becthold JE, Kold S, Soballe K.
J.Orthop.Res.; 2008: Accepted.

The papers will be referred in the text by their Roman numerals (I-III).

Study I was given the Best Poster Award by The Danish Orthopaedic Society at the annual spring meeting, Aalborg, 2005, and the Best Poster Award by The Faculty of Health Sciences, Aarhus University, at the annual PhD day, 2006.

Study II was given the Best Poster Award by The Faculty of Health Sciences, Aarhus University, at the annual PhD day, 2008.

Supervisors:

Kjeld Søballe, Professor, MD, DMSc
Department of Orthopaedics
Aarhus University Hospital, Denmark

Søren Kold, MD, PhD
Orthopaedic Research Laboratory
Department of Orthopaedic,
Aarhus University Hospital, Denmark

Ole Rahbek, MD, PhD
Orthopaedic Research Laboratory
Department of Orthopaedic,
Aarhus University Hospital, Denmark

Joan E. Bechtold, PhD
Midwest Orthopaedic Research Foundation
Minneapolis, USA

Correspondence:

Thomas Jakobsen, MD
Orthopaedic Research Laboratory
Department of Orthopaedics
Aarhus University Hospital
Norrebrogade 44, Build. 1A
Dk-8000 Aarhus C
Denmark
Email: Thomas.jakobsen@ki.au.dk

Preface

This PhD thesis is based on scientific work conducted during my enrolment as PhD student at the faculty of Health Sciences, Aarhus University, from 2005-2008. The experimental work was performed at Orthopaedic Research Laboratory, Aarhus University Hospital, and Orthopaedic Biomechanics Laboratory, Hennepin Medical County Center, Minneapolis, USA. The studies are a continuation of the work performed during my enrolment as research year student at the Health Sciences, Aarhus University, from September 2003 - August 2004.

Science can only be achieved by working as a group. The present studies could not have been done without the help from numerous people. I would like to thank my supervisors for making it all possible and my co-authors for their help and advises. A special thank to Jørgen Baas for sharing his profound knowledge. I also thank our highly skilled laboratory technicians for their expertise. This work could not have been possible without help from the staff at The Orthopaedic Biomechanics Laboratory, Minneapolis; I thank them for helping conduct the surgeries and for taking care of the animals. I would like to thank all PhD students and medical students for making The Orthopaedic Research Laboratory a wonderful place to do research; I especially enjoyed the discussions at our journal clubs. Finally, my deepest thanks go to Tine and my son, Anders, for keeping me on the right track.

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Contents

Summary

The number of total hip replacements is increasing, with the highest increase among people aged 50-59 years. Unfortunately, the failure rates for young patients are also among the highest. An improvement in implant longevity is needed.

Studies using radiostereometrical analysis (RSA) have shown that early prosthetic migration is associated with increased risk of aseptic loosening. One way to increase implant longevity could be an improvement of early implant stability.

A potential way to enhance early implant fixation could be with the use of bisphosphonates. These drugs are strong inhibitors of osteoclastic bone resorption. They are currently used against osteoporosis and osteolytic tumors. Several clinical and experimental studies have investigated the use of bisphosphonates as adjuvants in total joint replacements. The results are promising. The bisphosphonate used in the present studies was alendronate.

The aim of the studies in this PhD thesis was to improve implant fixation of experimental implants using alendronate as a local adjuvant. Implant fixation was defined in term of biomechanical stability and osseointegration.

Study I investigated the effect of local alendronate treatment on implant fixation of porous-coated titanium implants inserted with the use of bone compaction. Implants were inserted with the use of bone compaction into undersized cavities that had been radial enlarged, thus transforming the surrounding bone into a zone compacted autograft. Implants were inserted bilaterally into the proximal part of tibia in ten canines. Alendronate was applied on one of the sides and saline on the other side. The observation period was 12 weeks. Push-out testing showed that alendronate increased the biomechanical fixation twofold. Histomorphometrical analysis showed that alendronate increased the amount of bone around and in contact with the implants.

Study II investigated the effect of soaking morselized allograft in alendronate before impacting it around a porous-coated titanium implant. In 10 canines, a pair of implants surrounded by a 2.5-mm gap was inserted into the proximal part of humerus during two surgeries separated by time. The gap was filled with allograft soaked in either alendronate or saline. The two implant pairs were observed for 4 and 12 weeks respectively. Push-out testing showed that alendronate dramatically decreased biomechanical implant fixation, and histomorphometrical analysis showed that alendronate almost blocked new bone formation and preserved the allograft.

Study III investigated the effect of local alendronate treatment on implant fixation of hydroxyapatite-coated implants inserted with the use of bone compaction. Study III had a similar design as used in study I. Push-out testing showed that local alendronate treatment was able to increase the biomechanical implant fixation. Histomorphometrical analysis showed that alendronate could increase the amount of both woven and lamellar bone around the implant, but not in contact with them.

The studies in this PhD thesis demonstrate that alendronate can increase fixation of implants inserted with the use of bone compaction. However, they also indicate that caution should be taken when using bisphosphonate as an adjuvant in allografted implants. The results warrant further preclinical investigation.

Introduction

The annual number of total hip arthroplasties (THA) has increased in Denmark over the last two decades [1]. In 2006, 9348 THA were preformed in Denmark, 15% of these were revisions [2]. The same tendency is seen in other OECD counties [3]. Based on numbers from the Danish Hip Arthroplasty Register and the StatBank Denmark the incidence of THA in Denmark is expected to increase with 210% from 2002 to 2020 [1;4]. This emphasizes the need to enhance the capacity for primary THA surgeries.

A strong predictor of long-term implant failure after primary THA is young age [5]. In addition, the highest increase in THA incidence can be found among patients aged 50-59 years [1]. The combination of increased risk of long-term implant failure among young patients and a high incidence increase in this group implies that a relative large increase in the revision burden can be expected. More than 70 % of all revisions are due to aseptic loosening of the prosthesis [4]. This emphasizes the need to improve THA longevity.

One way to improve THA longevity could be through an improvement of the early implant stability. Several studies have investigated the association between early implant migration and long-term implant failure using radiostereometrical analysis (RSA) [6;7]. Kärrholm et al. found that the probability of revision after seven years was greater than 50% if femoral stem subsidence at two years was 1.2 mm or more [6]. The causal association between early migration and long-term aseptic loosening is still unknown. There are, however, increasing evidence that the etiology is multifactorial [8]. It seems likely that initial micromotion of the implant opens up the interface to joint fluid and wear particles through the creation of a fibrous membrane around the implant [9;10]. The presence of wear products at the bone-implants interface is believed to be a strong activator of macrophage induced bone resorption [11]. The hypothesis that a fibrous membrane around the implant increases transportation of wear particles from the joint space to the bone-implant interface, and thus promoting osteolysis, is supported by the fact that sealing of the interface with the use of a hydroxy-apatite (HA) coating decreases particle transportation [12;13]. HA-coatings have been shown to create a tight bonding between implant surface and surrounding bone [14]. The importance of early micromotion in the process of implant failure emphasizes the need to optimize early implant osseointegration and stability.

Aim

The overall aim of this PhD thesis was to increase primary THA longevity and thereby reduce the risk of painful implant failure and costly revision arthroplasty. The specific aim of this PhD thesis was to facilitate osseointegration and enhance early biomechanical implant fixation, and thereby, hopefully, contribute to an increase in THA longevity. The studies in this PhD thesis investigated whether local treatment with bone anti-resorptive drugs, bisphosphonates, could increase implant fixation and osseointegration. All experiments were conducted with experimental implants placed in canine cancellous bone. Implants were either inserted with the use of bone compaction or surrounded by impacted allograft. Common for all studies was the use of local bisphosphonate treatment. Biomechanical implant fixation and implant osseointegration were evaluated with the use of push-out test and histomorphometry.

Hypotheses

I:
Local bisphosphonate treatment can increase biomechanical implant fixation and osseointegration of experimental implants inserted with the use of bone compaction (Study I and III).

II:
Impacting morselized allograft soaked in bisphosphonate around experimental implants can increase biomechanical implant fixation and osseointegration, and reduce allograft resorption (Study II).

Background

The story of total hip arthroplasty

Osteoarthritis is a chronic disease characterized by pain and reduced mobility. Over the last three centuries, surgeons have tried to treat this crippling disease. Some of the first attempts to treat osteoarthritis involved amputation of the leg or joint excision. Anthony White (1782-1849) from the Westminster Hospital in London was credited for the first excision arthroplasty in 1821 [15]. The success of the early arthroplasties was very limited. However, the need to reduce the deliberating symptoms from the disease was still imminent. The search began for materials that could be utilized to resurface or even replace the hip. One of the pioneers within interpositional arthroplasty was Léopold Ollier from Hôtel-Dieu hospital in Lyon, France. He described how to interposition adipose tissue into the hip joint. However, he did not fixate the adipose tissue to the bone and his procedure never became a success. In the following years many trails with different materials such as chromatized pig bladders, rubber struts, silver plates, and fascia latae were carried out. They were all meet with failure [16].

A large improvement in interpositional arthroplasty was made in 1923 by the Norwegian-born American surgeon Marius Smith-Petersen (1886-1953) [17]. He had during one of his surgeries excised a piece of glass surrounded by a smooth membrane of soft tissue. This finding leads him to mold a piece of glass, which could fit over the femoral head and provide a new smooth surface for movement. Due to the brittle nature of the molded glass the treatment never becomes a success. Facilitated by his dentist he changed the molded glass to Vitalium®, a newly developed cobalt-chromium alloy used in dentistry, and his mold arthroplasty provided the first good predictable results in hip arthroplasty.

Parallel with the development of interpositional arthroplasty, surgeons were trying the find ways to replace the diseased joint. The first attempt to perform a total joint replacement was carried out in 1891 by the Berliner Professor Themistocles Glück (1853-1952) [16]. He performed the joint replacement with an ivory ball and socked. The pursued to optimize joint replacement continued. The success was limited because most of the implants loosened from the bone. The problem was solved in 1958 by a very innovative English surgeon. He changed the material for the acetabular socked from metal to polyethylene and fixated the components with polymethylmetacrylate, also known as bone cement among dentists. The surgeon is today known as Sir John Charnley, and is credited for given birth to today's total hip arthroplasty (THA) [18].

Today´s total hip arthroplasty

Approximately 15 years after the introduction of bone cement in THA by Sir John Charnley a renewed focus was attended toward the problems with prosthetic loosening and peri-implant osteolysis. It was assumed that the sole cause for the implant failure was cement particles, hence the term "cement disease" [19;20]. Focus was once again on improving uncemented THA. A major improvement was introduced in 1968, where cobalt-chromium alloy implants were porous coated, thus allowing bone to growth into the implant surface [21]. The ingrowth of bone into the implant surface is today known as osseointegration [22]. In 2006, 47% of all THA in Denmark were uncemented, 31% were cemented, and 22% were hybrids [2]. Reports from the Norwegian Arthroplasty Register shows that uncemented femoral stems perform better than cemented ones in patients younger than 60 years [23]. However, there is still a need to improve the longevity of uncemented femoral stems in young patients, since being less than 60 years old is a strong predictor of long-term implant failure [5].

Metals for uncemented femoral stems

The implants are usually made of a different titanium (Ti) alloys, commercially pure (c.p.) Ti or cobalt-chromium (CoCr) [24]. The implants can either by cast or wrought. Ti-alloy and c.p. Ti implants have an elastic modulus closer to that of cortical bone compared to CoCr implants [25]. This may reduce the stress shielding around the implant. Furthermore, Ti-alloy and c.p. Ti implants are more corrosive resistance and biocompatible than CoCr implants. Ti-alloy implants are more corrosive resistance than c.p. Ti-implant, but less biocompatible [24;26]. CoCr implants are known to be the most wear and fatigue resistance implants. Stainless steel has been used for uncemented implants, but without success [27]. The implants used in this PhD thesis were Ti-alloy implants.

Surface treatments

The first uncemented implants had a smooth surface. They had an unacceptable failure rate and their use was abandoned in the early 1990s [23]. The uncemented implants used today all have a roughened surface applied by grit-blasting, etching, or porous coating. The term "porous", meaning hole, refers to a series of interconnected pores located on the implant surface. The pores are created by coating a layer of small particles onto the implant surface. The three most common techniques for porous coating are plasma-spraying, sintering bead technique, and diffusion bonding:

Plasma spraying is a technique where a heated metal powder is sprayed onto the implant surface.

Sintering bead technique bonds small beads to the implant surface by heating up the implant and beads [21].

Diffusion bonding is a technique where a fiber mesh made of small Ti wires is molded onto the implant surface with the use of heat and compression.

The implants in the PhD thesis were all porous coated with the use of plasma spraying.

Hydroxy-apatite coatings

Hydroxy-apatite (HA) is the most abundant mineral in bone. In 1987, de Groot demonstrated how to plasma-spray HA onto an implant surface [28]. Today, second generations HA coating exits, where the HA is precipitated onto the surface. HA coatings are often applied to porous implant surfaces, and are considered to be a bioactive coating with osteoconductive properties [29;30].

Several experimental studies have demonstrated superior properties of HA [26;29;31]. It has been shown that HA can increase osseointegration and biomechanical fixation of implant subjected to both stable and unstable conditions, and enhance bone across a gap. Furthermore, HA has been shown to convert a fibrous membrane, created around an implant subjected to micromotion, to bone. A property not seen with non-HA coated implants.

The clinical results with HA-coating are promising [32-35]. The general findings are excellent implant survival and reduced migration compared to non-HA-coated implants. However, not all studies are able to demonstration a reduced risk of implant failure when using HA-coated implants [36].

Biology of implant fixation

An implant can be fixated to bone by two different methods. The first method involves the use of bone cement as filler between the implant and bone. The fixation is dependent on the mechanical properties of the implant-cement-bone interfaces. The second method is biological and dependent on bone ingrowth into the implant after placing the implant in initial press-fit with the surrounding bone.

The regeneration of bone around an uncemented implant is in many aspects similar to fracture healing. Immediately after implant insertion, an inflammatory response is elicited. Due to vascular endothelial damage a hematoma will form around the implant. Blood circulation around the implant will be very limited the first days after implantation. Platelets in the hematoma

will release growth factors and contribute to formation of a blood clot. Cells from the immune system will be attracted to the implantation site by chemotaxic signals from the platelets and activated to release cytokines incl. bone morphogenetic proteins (BMP) that stimulate bone regeneration [37-39].

The inflammatory phase is followed by a reparative phase. Precursor cells differentiate into osteoblasts, than begin to form woven bone through the process of intramembranous ossification. If the bone-implant construct is rigid and without micromotion, then bone can form directly from the vital parts of the surrounding bone bed. Parallel with bone formation is osteoclastic resorption of necrotic bone generated by the surgical trauma. The bone formation and resorption is spatial and temporal. A lag time during the initial 4 weeks of healing, where no increase in torsional fixation was observed, has been shown experimentally in rodents [40]. This period corresponds with the presence of inflammation and removal of traumatized tissue.

The remodeling phase is the final phase in bone regeneration. Basic multicellular units (BMU) resorp the woven bone and lay down new lamellar bone. The activation-resorption-formation frequency of the BMU is increased in a fracture site compared the normal bone [37;38].

The ultimate goal when inserting an uncemented orthopaedic implant is *osseointegration*. The term "osseointegration" was first described by Brånemark in 1977 and later defined by Albrektsson as direct contact at the light microscope level between living bone and implant [22;41]. The definition of osseointegration implies that only histology can be used to evaluate whether an implant is osseointegrated. Due to the limited clinical application of the histological definition a biomechanical definition has been suggested: "A process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading" [42]. The degree of rigid fixation can be evaluated by radio-stereometric-analysis (RSA).

Implant osseointegration is dependent on a variety of factors. An important prerequisite for osseointegration is *osteoinduction*. The term "osteoinduction" describes the process were primitive, undifferentiated and pluripotent cells are induced to develop into the bone-forming lineage. Osteoinduction can by defined as: "the process by which osteogenesis is induced"[43]. The presence of bone precursor cells is necessary for *osteogenesis*. Strong osteoinductive factors are the BMP. These glycoproteins, with the first being discovered by Urist in 1965, have the capacity to induce heterotopic osteogenesis [44;45]. BMP are naturally released in response to trauma, e.g. implant insertion, and are the only known inductive agents [46]. Bone healing is dependent on osteoinduction induced by the release BMP and subsequent differentiation of bone-forming cells.

Another important factor for osseointegration is *osteoconduction*. The term "osteoconduction" means that bone grows on a surface. The surface can originate from an implant or a graft material such as bone allo- or autograft. The osteoconductive material can be regarded as a passive scaffold onto which new bone is formation. A prerequisite for osteoconduction is osteoinduction [37;38]. Furthermore, the degree of osteoconduction is in part determined by the biocompatibility of the material. The impact of biocompatibility on osteoconduction can be illustrated when studying the significant different amounts of bone that grows on different metal surfaces such as c.p. Ti and Ti-alloys [24;47].

Biology of bone grafts

Ideally, an implant should function at optimal level throughout the life of the patient. However, this is rarely the case and most implants do not survive indefinitely. When an implant fails, bone stock is diminished due to osteolysis [48]. One way to restore the bone stock is with the use of bone graft. The method was first described in 1975 by Hastings and Parker [49]. The use of impacted morselized bone graft in conjunction

with cemented THA was developed by Slooff and Ling in 1984 and 1991 [50;51]. Their technique is known as impaction bone-grafting.

Various types of non-synthetically bone grafts exist. Bone graft materials harvested from the same individual is referred to as autograft, while bone graft from a genetically different individual is called allograft. Bone graft from another species is called xenograft. A bone graft material can further be characterized as cortical, cancellous, corticocancellous, or osteochondral according to its appearance [52]. Allografts are usually modified or preserved to reduce immunogenicity before transplantation. These modifications include freezing, freeze, drying, irradiation, rinsing or chemomodification [53].

The objective when using impacted, morselized allograft in conjunction with THA is to achieve mechanical implant stability while allowing the restoration of living bone stock by bone ingrowth. The initial mechanical stability is achieved by impacting bone chips as large as possible with a low fat content into the medullar canal or acetabulum. The goal is to create a compacted bone bed with a high density [54]. Long-term mechanical implant stability is depending on graft incorporation. The process of graft incorporation is biological and describes an interaction between the graft material and host bone that results in bone formation and full or partial replacement of the graft leading to adequate mechanical implant stability [55].

Bone ingrowth into the morselized allograft can be facilitated by different mechanisms [56]: *Osteoinduction*; growth factors such as BMP embedded in the graft are released and stimulate local bone formation. Due to post-harvesting treatment, autograft has a larger osteoinductive potential than allograft. *Osteoconduction*; the surface of the graft acts as a scaffold for bone formation. The degree of osteoconduction is influenced by the relative area of surface pr. volume, e.g. cancellous graft exerts a higher degree of osteoconduction than cortical graft. However, increased degrees of graft density can reduce osteoconduction [57]. This can be explained by the immpacted graft acting as a hindrance for ingrowth of tissue. *Mechanical loading*; transfer of mechanical load through bone allograft stimulates new bone formation. This has been shown in various animal models [58;59]. The result of these mechanisms is formation of bone within the graft. Studies suggest that bone formation within mechanically stable grafts occurs as intramembranous ossification [55;60].

The interaction of osteoconduction and osteoinduction is necessary for graft incorporation. This interaction ultimately leads to the replacement of the graft by host bone under the influence of load bearing [52]. The process by which allograft is replaced by new bone is known as creeping substitution [55]. This process is, as remodeling, coupled and dependent on both osteoclasts and osteoblasts.

The mechanical strength of cancellous bone graft increases as new bone is formed. However, if bone resorption exceeds bone formation, then the mechanical implant stability can be compromised. A stimulus for resorption could be stress-shielding.

The bone compaction technique

Numerous studies have shown the importance of initial implant stability for osseointegration of cementless implants [14;61-63]. Secondary implant stability and long-term survival cannot be achieved without proper implant osseointegration. Initial implant stability can be enhanced by placing the implant in close-fit with the surrounding bone [64;65].

One way to improve the initial implant stability could be with the use of the bone compaction technique. In THR, the bone compaction technique sequentially expands cancellous bone using increasing sizes of smooth tamps before implant insertion [66]. This is in contrast to conventionally rasping where bone is partly removed.

Bone compaction was first investigated experimentally by Channer et al. in 1996 [67]. They found in a human cadaver study that the

stability of a cementless tibia stem was significant higher than conventional press-fit. Increased mechanical fixation was also found in a human cadaver model of THR when comparing bone compaction to rasping [66]. However, two cadaver studies have found increased risk of per-operative fracture when preparing a femur for implant inserting with bone compaction [68;69].

In vivo canine studies have shown that bone compaction increases the mechanical fixation of experimentally porous-coated Ti and HA implants [70-73]. Furthermore, the same studies showed that bone compaction was able to increase both the amount bone of around and in contact with the implant. Some of this bone was by appearance traumatized and non-vital. A concern about the mechanical implant stability during the resorption of this non-vital bone was raised. A study with a longer follow-up period showed no adverse effects on implants stability during resorption of the non-vital bone [74].

The increased implant fixation as a result of bone compaction can be explained by several causes. Bone is known to be a visco-elastic material [75]. It has been shown that compacted bone has a spring-back effect and an ability to reduce initial gaps between bone and implant [76]. Due to the visco-elastic properties of bone, implants inserted with the use of bone compaction can be considered to be placed in extreme-fit. Another property of the bone compaction technique is the creation of zone around the implant consisting of compacted fractured bone [70]. This zone can be considered as bone autograft created in situ, and might facilitate new bone formation.

Bisphosphonates

Bisphosphonates have been known to chemists since the mid 19th century. They were mainly used in textile, fertilizer, and oil industries to prevent scaling because of their inhibitory properties on calcium carbonate precipitation. The biological effects of bisphosphonates were discovered in 1968, where Fleisch et al. found that analogues of inorganic pyrophosphate could prevent formation and dissolution of calcium phosphate *in vitro* [77]. Inorganic pyrophosphate had previous been shown to have the same properties *in vitro*, but limited therapeutic use *in vivo* due to rapid enzymatic hydrolysis [78]. Bisphosphonates are analogues of inorganic pyrophosphate, which can resist enzymatic hydrolysis and metabolism.

Bisphosphonates are compounds characterized by two C-P bonds on the same carbon atom (P-C-P) instead of the P-O-P bond of inorganic pyrophosphate. The biological characteristics of a bisphosphonate can be modified by changing the side chains. Many bisphosphonates are commercially available as inhibitors of bone resorption and are used in the treatment of bone disorders such as osteoporosis, tumor bone disease and morbus Paget.

Pharmacokinetics of bisphosphonates

The oral bioavailability of bisphosphonates in animal and humans is low. Using a double-isotope design, the oral bioavailability of alendronate, the bisphosphonate used in this PhD thesis, has been estimated to 1.8% and 0.6% in dogs and humans respectively [79]. The poor intestinal absorption is likely attributed to the low lipophilicity of bisphosphonates, and their negative charge. Between 30-70% of the bisphosphonate in plasma are taken up by the bone, the remainder is being excreted rapidly into the urine [79]. More than 50% of absorbed alendronate is taken up by the bone. The half-life of circulating bisphosphonate is estimated to be around 0.5-2 hours in humans, and in the order of minutes in rats [80]. Bisphosphonates are resistant to enzymatic degradation, and are not metabolized in the body [80].

Bisphosphonates bind preferentially to bone tissue with high turnover rate and their distribution in bone is not homogeneous [81-83]. The preferred binding site in bone is surfaces undergoing resorption, and secondary surfaces with bone formation. The preference for resorptive surfaces could be explained by the high affinity of bisphosphonates to hydroxyapatite at

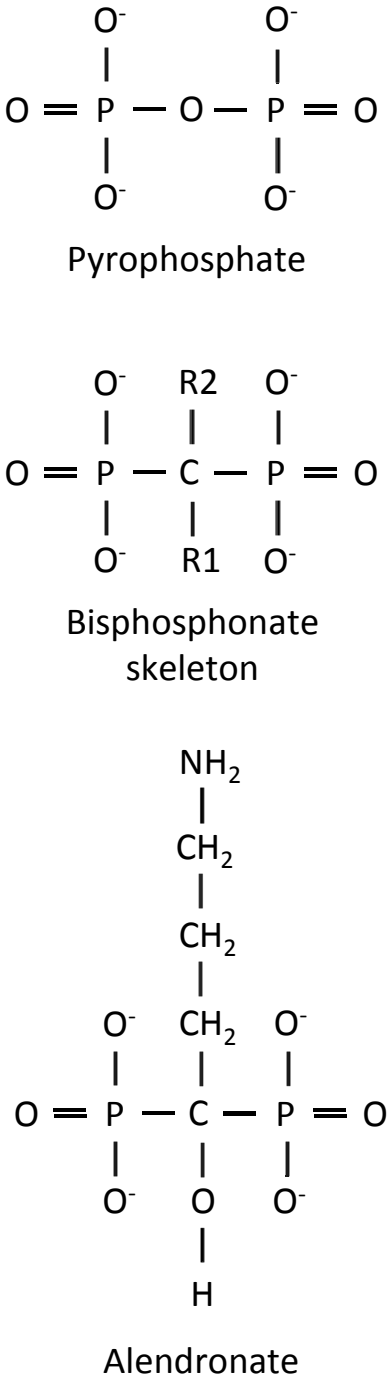


Fig. 1: Chemical structures of pyrophosphate, germinal bisphosphonate and alendronate.

physiological pH [82;84]. This could also explain why bisphosphonates, in therapeutic doses, only exert their effects on bone. Furthermore, the high affinity for hydroxyapatite makes bisphosphonates an ideal candidate for topical treatment of bone with relative high amount of hydroxyapatite exposed surfaces. Such surfaces can be found on morselized bone graft and on the microchips created by the bone compaction technique. Bisphosphonates are released from bone during bone resorption due to acidic milieu in the subosteoclastic space and are subsequent internalized by the osteoclast by endocytosis [82]. This might explain why bisphosphonates primary affects osteoclasts. Bisphosphonates bond to or build into bone can be considered pharmacological inactive. The half-life of alendronate in bone equals bone turnover and is estimated to be 3 years for dogs and 10 years for humans [79].

Actions on the molecular and cellular level

There are two general classes of bisphosphonates: those that form toxic analogues of ATP and those that inhibit the farnesyl pyrophosphate synthase (FPP synthase) [85;86]. The presence or absence of a nitrogen atom in the R^2 side chain determines the mechanisms of action. Those that contain nitrogen inhibit the FPP synthase and are called N-bisphosphonates, while the non-N-bisphosphonates form toxic ATP analogues [87;88]. Alendronate is an N-bisphosphonate.

The FPP synthase is an enzyme in the mevalonate pathway and is necessary for the formation of isoprenoid lipids such as farnesylpyrophosphate and geranylgeranyl-pyrophosphate (Fig. 2). These lipids are required for post-translational modification of GTP-binding proteins such as Ras, Rho, Rac and Rab. These proteins are important for regulation of cell growth, differentiation, survival, vesicular trafficking and cytoskeletal organization [89-91].

At the cellular level bisphosphonates has been shown to inhibit osteoclast recruitment and activity, shorten lifespan and adhesion to bone [89;92-94]. The mechanisms behind these effects are still unclear, but some experiments attribute the effects to the lack of isoprenoid lipids[95]. There is a good correspondence between the inhibitory effect on the farnesyl diphosphate

synthase by a bisphosphonate and its inhibitory effect on bone resorption.

Some *in vitro* studies indicate that bisphosphonates can stimulate proliferation of osteoblasts and might enhance bone formation [96;97]. These findings are still to be reproduced *in vivo*.

Effects on bone

Bisphosphonates inhibit bone resorption in both normal animals and in animals with stimulated hyperresorption [98]. As a result the bone mineral content and calcium balanced is increased due to a filling up of the remodeling space and an increase in intestinal absorption of calcium as a consequence of elevated level of $1,25(OH)_2$ vitamin D [99]. Furthermore, bone formation is decreased due to the coupling between the osteoclast and osteoblast in BMU. The overall effects are a decrease in bone turnover and increase in bone density.

The effect of bisphosphonates on the mechanical properties of bone has been investigated in both experimental and clinical studies [100-103]. The general finding was a conservation of bone strength. However, prolonged administration of high doses could reduce bone turnover and impair healing of microscopic cracks. This could result in accumulation of microdamage, which subsequent could impair bone strength [104;105]. In a clinical study with ten year follow-up no significant decrease in incidence of fractures could be found [106]. In a rodent model of fracture healing relative high doses of incadronate has been shown to increase callus size and postpone final repair, but increase the mechanical strength [107]. The same results have been observed in a canine study [108]. A clinical study investigating the effect of a yearly infusion of zoledronate after a low-trauma hip fracture found an increased survival and a reduction in the rate of new clinical fractures [109]. Although these data are encouraging, there is still a need to study the long-term effects of bisphosphonates on damage accumulation, architecture, and mechanical properties.

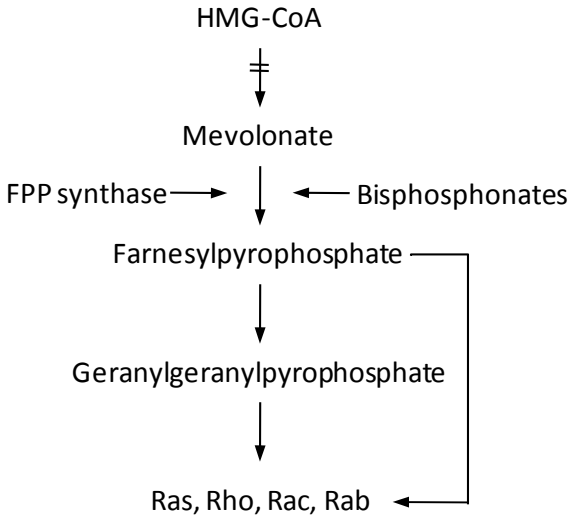


Fig. 2: Molecular action of nitrogen-containing bisphosphonates on the pathway leading from mevalonate to post-translational modification of GTP-binding proteins (Ras, Rho, Rac, Rab). FPP = farnesyl pyrophosphate.

Bisphosphonates in the context of THA

The anti-resorptive properties of bisphosphonates have shown encouraging results in the context of THA. Experimental studies indicate that particle induced osteolysis can be inhibited with both local and systemic administration of bisphosphonate [110;111]. Clinical studies show that systemic administrated bisphosphonates can reduce bone loss associated with stress shielding [112;113].

A strong predictor for long-term implant survival is early osseointegration and stability. Several experimental studies have investigated the effects of bisphosphonate treatment on implant fixation [114-118]. The general findings are increased implant osseointegration and mechanical stability. Furthermore, clinical studies have demonstrated that peri-operative treatment with bisphosphonate, either local or oral, was effective in reducing tibial component migration in cemented total knee arthroplasty [119;120]. The migration was measured with RSA.

Another interesting feature with bisphosphonates is their ability to preserve bone grafts while increasing new formation within in the graft [121-123]. These results indicate that

bisphosphonate, at the same time, can facilitate graft and implant osseointegration while protecting the graft against resorption until new bone has formation and reinforced it mechanically. The allograft preserving properties of bisphosphonate has also been shown clinically in patients receiving a cemented THA [124].

Material and methodological considerations

Experimental models

Experimental animals

Various experimental animal models have been used in the context of total joint replacement [125]. The choice of experimental animal model depends on the question raised. The dog is a common used animal in experimental models, where the focus is on implant fixation and osseointegration, and also the choice of animal for the studies in this PhD thesis. The dog is a large animal with a bone structure that closely resembles the human bone structure [126]. It has large bones which imply that several treatment groups can be tested in a paired design. Extensive research have been carried out at our institution using the dog as experimental animal [13;14;70;127-131]. However, the dog is expensive and more difficult to handle than rodents.

The dogs used the in present studies were all skeletally mature and breed for scientific purposes. Surgery and observation were conducted at Midwest Orthopaedic Research Foundation, Hennepin County Medical Center, Minneapolis, USA. All experiments were approved by the local Animal Care and Use Committee. Institutional guidelines for treatment and care of experimental animals were followed.

Design of studies

All experiments in this PhD thesis were designed as paired studies with control and intervention implants in the same animal. The paired design eliminates the contribution of the inter-individual variance to the total variance and reduces the number of animals needed to detect a given difference.

Symmetry between left and right implantation sites were assumed for study I, hence control implants was implanted in left tibia and intervention implant in the right tibia. The symmetry of the canine extremities has previously been described [132]. However, the study only describes the symmetry in geometrical properties and not e.g. symmetry in loading pattern. Study I is therefore limited since no alternation of treatment group was done between left and right implantation site. In study III, different treatment groups were alternated between the different implantation sites with random start. In study II, two implant pairs were inserted into each dog with one pair in each humerus. An implant pair consisted of a control and an alendronate implant. The implant pairs were observed for 4 and 12 weeks respectively. Implantation of implant pairs from the two observation periods was alternated between left and right humerus. Implantation of implant types (control or alendronate) within each implant pair was alternated between proximal and distal position. The design for study II implies that alendronate could affect the neighboring control and thereby diminish any potential treatment effect. An alternative design could be the placement of both alendronate in the same humerus and both control implants in the contralateral humerus. The drawback of this design is the repeated surgery on the same bone and thereby induction of a regional acceleratory phenomenon affecting the implant already in place [37]. Considering bisphosphonates's strong affinity to bone and thereby reduced risk of being transported to the control implant, the design with implants from the same observation period in the same humerus was considered most optimal.

Sample size

The number of dogs included in each study was calculated using the following formula:

$$n = \frac{(t_{1-\alpha/2} + t_{1-\beta})^2 x\ SD_{diff}^2}{d^2}$$

where:

n = number of animals

$t_{1-\alpha/2}$ = the (1-α/2) quantile in the t-distribution at two-sided testing

$t_{1-\beta}$ = the (1-β) quantile in the t-distribution at two-sided testing

SD_{diff}^2 = square of the standard deviation on the paired differences

d^2 = square of the minimal relevant difference

The risk of type I error (α) was set to 0.05 and the risk of type II error (β) was set to 0.20. Based on previous studies from our institution, the standard deviation (SD) on the relative difference was set to 50%. The minimal relevant difference (d) was set to 50% change in biomechanical implant fixation.

The quantiles in the t-distribution are dependent on the degrees of freedom. The number of animals needed (n) were calculated under the a priori assumption of ∞ degrees of freedom. This assumption results in the need of eight animals (n = 7.8) and 7 degrees of freedom. A new n was then calculated with the a priori assumption of 7 degrees of freedom. Continuing this approach until n and the degrees of freedom, for practical purposes, did not change anymore results in the need of ten experimental animals.

Implant models

Two different implant models were used in this PhD thesis. Common for both models was the transcortical implant placement in epiphyseal cancellous bone. The models were designed to imitate the portion of a cementless total joint replacement placed in cancellous bone. Both models are standardized, controlled and simple to reproduce, but limited by the lack of weight-bearing. The models are adapted from earlier studies conducted at our institution [70;118;128;133].

Bone compaction model (Study I and III)

The implants were inserted into the proximal part of tibia. Before implantation, the drill hole was locally treated with alendronate or saline, and then gradually expended from 5.0 mm to 8.0 mm (Fig. 3 and Fig. 5). The observation time for both studies was 12 weeks.

Care should be taken when evaluating the implant placement. The implant is intended to be surrounded by cancellous bone. However, if the medullar canal protrudes relative proximal, then some of the implant surface could potentially be without initially cancellous bone cover. X-rays were used to evaluate the placement of all implants.

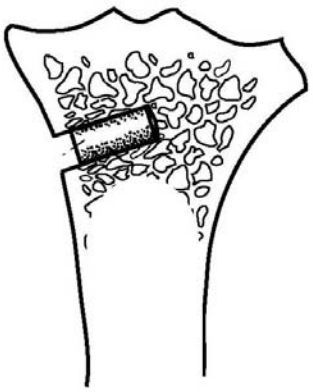


Fig. 3: Implant inserted into the proximal tibia

Allografted gap model (Study II)

Two implants were inserted into each proximal part of humerus. Each implant with a diameter of 6 mm was surrounded by a 2.5 mm circumferential gap obtained by attaching a bottom and top endcap with a diameter of 11 mm. The gap was filled with impacted morselized allograft soaked in either alendronate or saline.

The intimate placement of two implants in the same bone can constitute a potential bias. The implants could potentially influence each other leading to a different result than only one implant would have done.

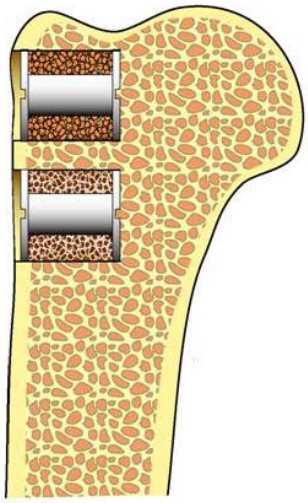


Fig. 4: Allografted implants inserted into the proximal part of humerus.

Implant characteristics

Implants for all studies consisted of custom-made titanium alloy (Ti-6Al-4V) core with a porous-coated titanium alloy (Ti-6Al-4V) surface deposited by plasma-spray technique. The implants for study III had an addition 50 µm plasma-sprayed hydroxy-apatite surface layer. All surface coatings were applied by Biomet Inc. (Warsaw, IN, USA). The roughness was not determined for the implants used in these studies. Manufacturer determined the mean pore size of the coating used in study II to 480 µm. Previous studies from our institution using the same surface coating reported a pore size of 200-1000 µm at the core and at the surface of the coating, respectively [31]. Furthermore, mean of departures from the roughness profile mean line (R_a) was determined to 47 µm for the plasma-sprayed titanium coating. The maximum peak to valley height (P_t) was measured to 496 µm. For HA-coated implants R_a and P_t were determined to 41 µm and 445 µm respectively [31]. Crystallinity of the HA-coating was determined by the manufacturer to 60%.

Surfaces coatings were applied using the same technique as on commercial available implants and are considered comparable to clinically used implants.

All implants were cylindrical of shape with a high of 10.0 mm and an outer diameter of 8.0 mm (study I and III) or 6.0 mm (study II). Endcaps with a diameter of 11.0 mm were attached to the implants used in study II.

Surgery

All surgery was done using sterile conditions and with the dogs under general anesthesia. Implantations sites were exposed using sharp dissection and periost was removed with the help of a rougine. A K-wire was used to guide the cannulated drill while creating the drill cavity. All drilling was at low speed with two revolutions per second to avoid thermal trauma to the bone. After implant insertion, the fascia and skin were closed in layers. All surgery was done by one person.

Unrelated studies were conducted in all three set of dogs used. The studies investigated the effects of different surgical techniques on loaded implants inserted into the medial femoral condyles or the effect of different surface coating on implants inserted into humerus or tibia. One study investigated the effect of local treatment with demineralized bone matrix on implant fixation.

Study I and III

A K-wire was inserted 20 mm distal to the tibia plateau. Over the K-wire, a cannulated step drill with a diameter of 5.0 mm the first distal 10 mm and 8 mm proximally was used to drill a 12.0 mm deep hole. Prior to surgery, 120 mg alendronate (MSD, West Point, PA) was dissolved in 60 mL saline. This alendronate solution was kept sterile at 5°C and used for all ten surgeries. In one knee, 5 mL of the alendronate solution (2 mg alendronate per 1 mL saline) was injected with a syringe into the hole for 60 seconds. The same amount of saline was used as control in the contra lateral knee. After soaking the bone for 60 seconds, excess bisphosphonate or saline solution together with blood coming from the marrow cavity was sucked away. The bone cavity was not irrigated. Next, the diameter of the 10.0 mm deep part of the hole was gradually expanded from 5.0 mm to 8.0 mm using custom designed compaction

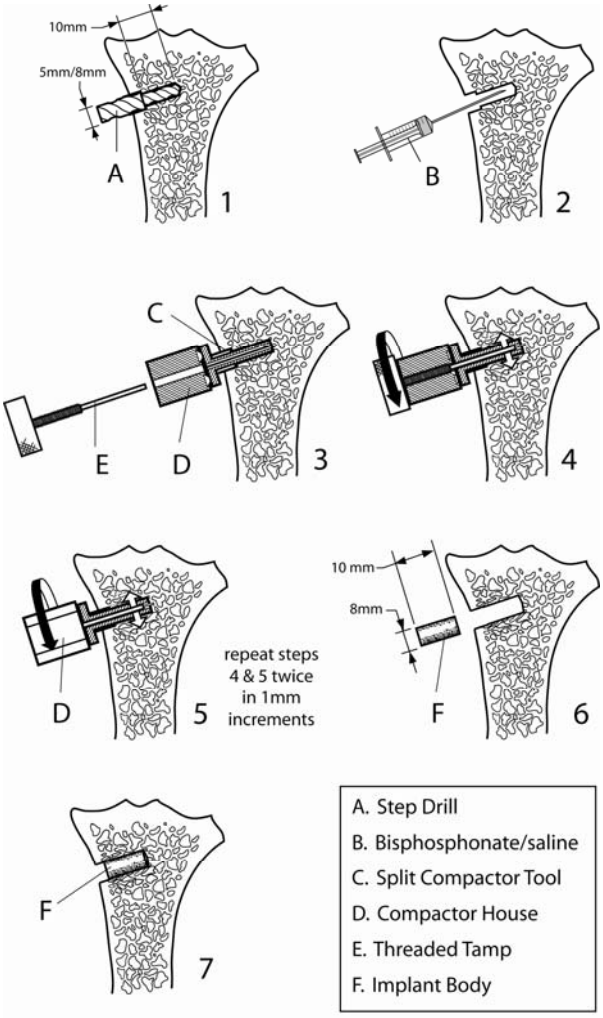


Fig. 5: The steps in the bone compaction technique. See text for description.

tools (Fig. 5). This resulted in a 12.0 mm deep hole with a diameter of 8.0 mm, where the diameter at the 10.0 mm depth was in part obtained by compaction and the diameter at the 2.0 mm superficial part was obtained by drilling. Immediately after compaction, the implant was inserted into the 10.0 mm deep part of the cavity.

Study II

The dogs were operated at two consecutive surgeries with 8 weeks between. Allograft for each dog was prepared in two different sessions, one before each surgery. Before implantation, allograft was soaked in either 5 mL saline or 5 mL alendronate solution (2 mg pure alendronate per milliliter; MSD, West Point, PA) for 3 minutes and then squeezed to remove excess fluid before being impacted into the peri-implant gaps. The allograft was not rinsing with saline before being impacted around the implants.

Two K-wires were inserted perpendicular in to the humerus surface with a 17.0-mm distance between them. The most proximal K-wire was inserted at the level of the greater tubercle. Over the K-wires, a 12.0-mm deep hole was made with an 11.0-mm cannulated drill. After removing bone debris and irrigating the bone cavity, the implant with a footplate was inserted. Morsellized allograft (± alendronate) was impacted into the 2.5-mm gap around the implants. The surgeon was not blinded to the type of allograft (± alendronate) he impacted.

Observation time

The choice of observation period depends on the question asked and the experimental model designed to answer this question. The aim of the studies in this PhD thesis was to improve the early implant fixation. If the observation period is too short, then there is a risk of a potential effective treatment not having time to exert its effect. If the observation period is too long, then there is a risk of not detecting a potential effect, since the control implant, although at slower speed, might be able to reach same implant fixation. Previous studies from our institution have shown that bone stimulation factors can enhance implant fixation and osseointegration in a canine implant model after 4 weeks [128;134]. Based on these results a 4-week study investigating the effect of local bisphosphonate treatment on implants inserted with the use of bone compaction was designed [133]. The study was able to demonstrate increased osseointegration due to bisphosphonate treatment, but not increased implant fixation. It was concluded that more time was needed for the treatment to be effective on implant fixation. The observation period for the studies in this PhD thesis is based on this conclusion.

An important issue to consider when facilitating early implant fixation is adverse effects. A treatment with a positive effect after 12

weeks is of little clinical use if the implant fixation is compromised within the e.g. initial 8 weeks and the patient is forced to reduced weightbearing. No data were available on the effect of alendronate on fixation of allografted implants in a canine model after 4 weeks. This consideration motivated the inclusion of a 4-week observation period in study III.

Specimen preparation

Two specimens containing the implant and surrounding bone were cut from each tibia or humerus perpendicular to the long axis of the implant using a water-cooled band saw (Exact Apparatebau, Nordenstedt, Germany)(Fig. 6). The first and most superficial specimen with a thickness of 3.5 mm was stored at -20°C pending biomechanical testing. The second specimen with the remaining part of the implant was fixed in 70% ethanol and embedded for later histomorphometrical analysis. Preparation of specimens was performed blinded.

The used preparation method and subsequent analyses dictate that biomechanical and histomorphometrical results are obtained from the different parts of the implant. This could potentially introduce a bias when correlation the biomechanical and histomorphometrical results. However, given the close relationship between the specimens, the change in bone quality between the two specimens is considered negligible, and thereby also the risk of introducing a bias.

Another way of introducing potential bias is the used method for storing the specimens for biomechanical testing. It has previous been shown that freezing can affect the viscoelastic properties of trabecular bone [135]. The changes were, however, small. It was also shown that defatting bone specimens could affect the viscoelastic properties. The use of relative paired changes in biomechanical implant fixation, instead of absolute values, reduces the impact of a potential bias due to freezing.

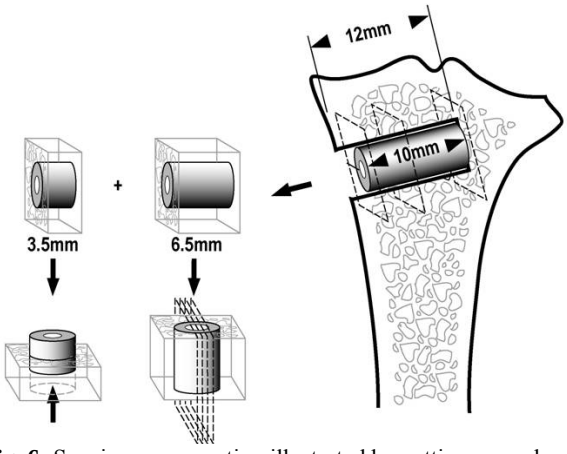


Fig. 6: Specimen preparation illustrated by cutting procedure of implant in tibia (Study I and III). Each bone-implant specimen is cut into two pieces: 3.5 mm for biomechanical testing, and a 6.5 mm for histomorphometry.

Biomechanical testing

The biomechanical implant fixation was tested by a destructive push-out test on an Instron Universal test machine (Instron Ltd, High Wycombe, UK) (Study I) or a MTS Bionics Test Machine (Study II and III) (MTS, Eden Prairie, MN, USA). Testing was done using a 10 kN load cell.

The bone-implant specimens were placed on a metal support jig with a diameter 1.4 mm larger than the implant diameter opening. Centering the implant over the opening assured a 0.7-mm distance between the implant and support jig as recommended [136]. Bone-implant specimens were thawed for one hour prior to testing. Testing was done blinded and in one session for each study. Implants were pushed from the peripheral side towards the inside of the bone. A preload of 2-3 N defined the start of the test. The test was conducted with a displacement rate of 5 mm/min, and continuous force versus displacement data were recorded (Fig. 7). These data were used to calculate parameters describing the biomechanical implant fixation. Reproducibility of push-out test was impossible due to its destructive nature. Reproducibility of the estimated biomechanical parameters was not preformed, since the estimated values were auto-generated.

Bone is known to be a viscoelastic material [75;137;138]. A viscoelastic material is one that

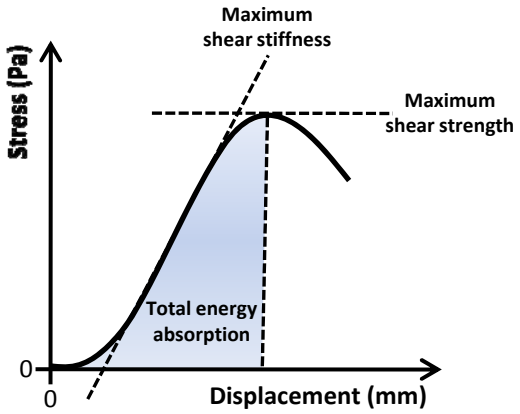


Fig. 7: Normalized stress-displacement curve.

undergoes material flow under sustained stress and exhibits different biomechanical properties under different rates of loading. The viscoelastic properties of bone can partly be explained by its content of water. This emphasizes the importance of all specimens being thawed before testing. Furthermore, in order to reduce the viscous component of stress under deformation, and thereby increase testing sensitivity, the displacement rate was chosen to be relative low.

The relative small opening of 0.7 mm around the implants in the support jig were chosen in order to optimize the evaluation of the biomechanical properties at the bone-implant interface and to see whether a potential increase in osseointegration were reflected biomechanically. Implant fixation is not only dependent on adhesion/interlock between bone and implant surface, but also on high quality bone further away from the bone-implant interface. A strong bone-implant interface is of little use if the supporting bone further away from the implant surface is of relative low quality. The optimized biomechanical evaluation of the bone-implant interface is therefore at the cost of lost information about the biomechanical properties of the bone peri-implanteric bone (Fig. 8).

A potential overestimation of biomechanical implant fixation can be introduced if the bone-implant specimen is not cut perpendicular to the long axis of the implant (Fig. 9). This will result in increased load needed to

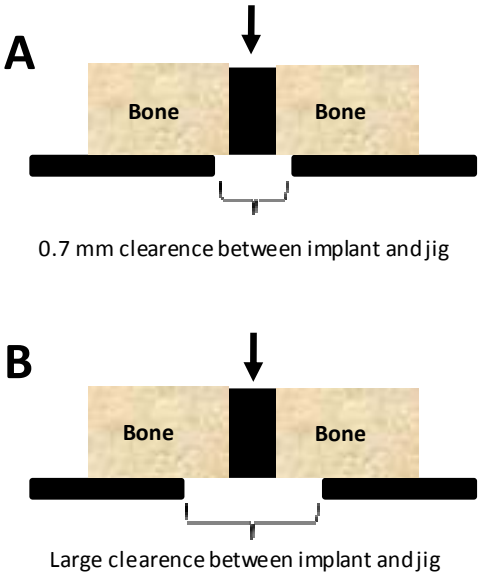


Fig. 8: Bone-implant specimen placed on supporting jig before push-out testing. Arrow indicates direction of displacement. Bone-implant interface is tested in situation "A", while situation "B" also includes testing of the bone further away from the interface.

displace the implant due to the supportive bone under the implant and a relative increase in bone-implant interface compared to specimens with same height. It is assumed that specimens not cut exactly perpendicular to their long axis are distributed random between the different treatment groups, and that they do not constitute a potential bias.

Biomechanical parameters

The specimens had various heights and the implants in the specimens had various diameters (Table 1). In order to reduce the impact of these geometrical variances on the total variation, force-data were normalized by the implant surface area. Implant surface area was calculated as:

Implant height x outer implant diameter x π

The used normalization transforms force-data to stress-data. Using stress-displacement curves, three biomechanical parameters were calculated:

- Maximum shear strength (MPa)
- Maximum shear stiffness (MPa / mm)
- Total energy absorption (kJ / m^2)

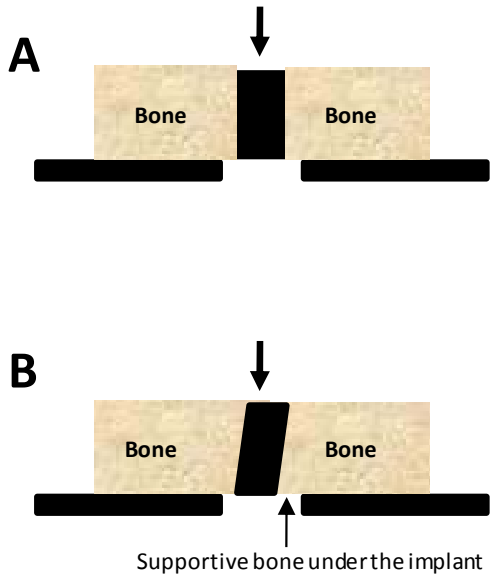


Fig. 9: Increased force is needed to displace an implant not cut perpendicular to its long axis (B). This is due to supporting bone under the implant and a relative larger implant surface as compared to an correctly cut implant (A) in a specimen with the same height. Large arrows indicate direction of displacement.

Maximum shear strength was defined as the first local peak on the stress-displacement curve. The first local peak was regarded as biomechanical failure at the bone-implant interface. Maximum shear stiffness was calculated as the maximum slope between five successive points of the elastic part of the force-displacement curve. Total energy absorption was calculated as area under curve until failure (Fig. 7).

The biomechanical implant fixation is illuminated by the three biomechanical parameters. These parameters are independent of each other and reflect different aspects of the implant fixation. The maximum shear strength reflects the stress the bone-implant interface can tolerate at the used displacement rate. Both mineralized and fibrous tissue can tolerance relative high stress forces before failure.

The maximum shear stiffness reflects the elastic modulus or the rigidity of the bone-implant interface at the used loading direction. Maximum shear stiffness is the most optimal parameter for identifying the predominant tissue at the interface, since different tissues have different modulus of elasticity. Mineralized tissue has a high elastic modulus, while fibrous tissue has a low elastic modulus.

Table 1. Sizes of implants used for push-out test

Study	Height (mm)	Diameter (mm)
I	3.78 (0.25)	7.56 (0.22)
II	3.22 (0.23)	5.85 (0.31)
III	3.22 (0.23)	8.03 (0.16)

Data are presented as mean (SD)

The total energy absorption reflects the energy needed to induce failure at the bone-implant interface and is a measure of the toughness. Two materials can have the same toughness with entirely different stiffness and strength.

The implant surface used for normalization represents a smooth cylinder. The implants used were all porous-coated with a relative higher surface area than a smooth cylinder given same height and diameter. The presented force-data are overestimated compared to the true values. This does not constitute a problem since data only are compared relative to each other.

Bone is known to have different mechanical properties when loaded in different directions [139]. This phenomenon is known as anisotropy of bones mechanical properties. This implies that potential different biomechanical values could have been obtained if the bone-implant interface had been tested under different conditions (e.g. pull-out test or torsional test). The used push-out test was chosen in order to imitate the stress, most often, applied to a joint implant *in vivo*. It should be noted that the used push-out test not only testes shear forces at the bone-implant interface, but also tensile and compressive forces of the bone interdigitating with the porous surface on the implant.

Biomechanical parameters in a clinical context

The ultimate goal of any adjuvant treatment in the context of total joint replacements is to optimize the longevity and prevent implant loosening. A prerequisite for successful implant survival is early osseointegration and stable fixation [6;7].

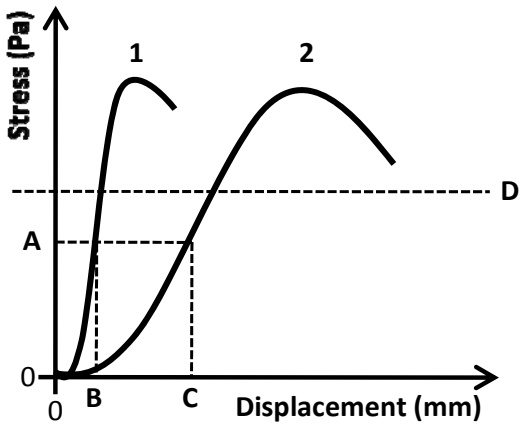


Fig. 10: A given stress (A) applied to an implant can result in two different magnitudes of movement (B or C) between the implant and bone depending on the stiffness of the bone-implant interface (1 or 2). Stress values below line (D) represent every day use of implants, while stress values above represent extreme use.

It seems reasonable to assume that the everyday stress applied to a total joint replacement is relative far away from point of implant failure on a stress-displacement curve. This reduces the need to improve strength and energy absorption.

As discussed in "Introduction", there is evidence that initial micromotion between the implant and bone will open up the interface for wear particles through the creation of a fibrous membrane around the implant [9;10]. The presence of wear particles is believed to be a strong activator of macrophage induced bone resorption and subsequent aseptic implant loosening [11]. During each gait cycle, stress forces are applied to the bone-implant construct. This stress will results in a micro-movement between the implant and bone. The magnitude of this movement is determined by the stiffness of the bone-implant interface. It is desirable to reduce to magnitude of this implant movement, since micromotion increases the risk of implant loosening. Increasing the stiffness of the bone-implant interface will reduce the magnitude of micromotion. Primary focus should be on improving the stiffness of the bone-implant interface.

Histomorphometrical analysis

Implant osseointegration was evaluated by histomorphometrical analysis. Specimens for histomorphometrical analysis were dehydrated gradually in ethanol (70–100%) containing basic fuchsin and then embedded in methyl-methacrylate. Four vertical uniform random sections were cut with a hard tissue microtome (KDG-95, MeProTech, Heerhugowaard, The Netherlands) around the center part of each implant as described by Overgaard (Fig. 11)[140]. Before making the sections, the implant was randomly rotated around its long axis. The sections were cut parallel to this axis. The 20-30 µm thick sections were cut with a distance of 400 µm, and counterstained with 2% light-green (BDH Laboratory Supplies, Poole, England) [141]. The penetration depth of light green into bone is 5-10 µm after 2 minutes of staining [142].

Histological examination and histo-morphometrical analysis was done using a light microscope (objective x10, ocular x10). Fields of vision from the microscope was transmitted to a personal computer monitor by a video camera attached to the microscope. Histomorphometrical analysis was performed using a stereological software program (CAST-Grid, Olympus Denmark A/S). The stereological software superimposes test probes on the field of vision from the microscope and enables the observer to estimate histomorphometrical parameters. The analysis was done blinded.

The different types of tissues were discriminated from each other based on their morphological appearance. Bone was stained green and easy to discriminate from other tissues. Bone was subdivided into woven and lamellar

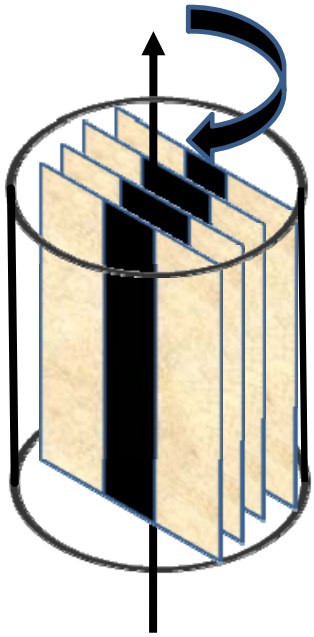


Fig. 11: Four vertical sections were cut parallel to the vertical axis of the implant. The implant was randomly rotation around the vertical axis before the sections was cut.

bone, and allograft. Woven bone had random orientation of osteocytes, large osteocytes and lacunae, and random orientation of collagen fibers whereas lamellar bone was arranged in parallel lamellae containing oval lacunae. In addition, polarized microscopy was applied to reveal the parallel lamellar structure when difficulties in discrimination between woven and lamellar bone were encountered. Allograft was lamellar bone without osteocytes in the lacunae. Fibrous tissue was stained red and identified by the presence of parallel fibers and low cell density. Bone marrow was stained red and identified by the presence of fat vacuoles and bone marrow cells. Fibrous tissue constituted less that 1% of the total tissue and was not subjected to statistical evaluation.

Implant osseointegration was evaluation in term of bone-to-implant contact and peri-implant bone density. The surface contact and peri-implant density were estimated as fractions of surface and volume with bone. Bone-to-implant contact was defined as bone in contact with the implant surface at the microscope level. The implant surface was easy to identify. Peri-implant bone volume fractions were estimated in a concentric zone either 1000 µm (Study I and III) or 2000 µm (Study II) around the implant. The size of these peri-implant zones of interest (PAI) was chosen to represent tissue were a potential treatment effect was expected to occur. The size of the PAI in study II was chosen to be 500 µm smaller than the concentric gap around the implant. This was done in order to avoid inclusion of tissue outside the gap in the PAI. As shown in table 1 the implant diameter was not exactly 6.0 mm. A PAI extending 2500 µm away from the surface of an implant with a diameter of more than 6.0 mm would include tissue from outside the gap.

Stereological principles

The objective with the histomorphometrical analysis was to evaluate the implant osseointegration in terms of bone-to-implant contact and peri-implant bone density. Surface and volume fractions are quantities present in 3D. The histological sections are in 2D. This implies that estimates of parameters present in 3D must be made from 2D histological sections. The use of stereology can help in the estimation of these parameters. The word “stereology” was coined in 1961 by the Foundation of the International Society of Stereology and defined as “spatial interpretation of sections”. Stereology is the science of estimating higher dimensional (e.g. 3D) information from lower dimensional (e.g. 2D) samples without introducing sampling and systematic bias.

Geometrical objects such as volume and surface fractions can be estimated from 2D sections with the use of probes (e.g. lines or points). The sum of dimensions for the probe and object must be three. In order to obtain an unbiased estimate the probes must by distributed uniform random and either the probe and/or object (e.g. volume density) must be isotropic. An isotropic object is an object with no preferred direction in space.

The basic stereological principles imply that volume fractions (3D) can be estimated using points (0D) as probes. A point is dimensionless, and therefore without any preferred direction in

space. Estimation of volume fractions does not require isotropy of the object of interest. Surface fractions (2D) can be estimated using lines (1D) as probes. The line is, as opposed to a point, not isotropic. Furthermore, a surface of an implant cannot be assumed to be isotropic. This means that neither the probe nor the object of interest is isotropic. This challenge can be overcome using the vertical sectioning technique. The technique was developed by Baddeley et al. in 1986 and allows estimation of surface fractions without assuming isotropy of the surface [143]. Four requirements must be followed when using this technique: 1) Identification of a vertical axis. 2) Uniform random rotation of the specimen around the vertical axis before sectioning. 3) Sections are cut parallel to the vertical axis. 4) The use of sine weighted test lines. These requirements assure that the test lines are isotropic and distributed uniform random in space (IUR). The vertical sectioning method assures estimates of surface fractions without systematic bias.

Stereological design

In order to obtain systematic unbiased estimates of surface fractions the sections were prepared according to the vertical sectioning technique as previously described. The long axis of the implants was used as the vertical axis, since it was easy to define at each step in the preparation process and subsequent histomorphometrical analysis. The specimens were embedded in cylindrical molds that allowed uniform random rotation of the implant around its vertical axis before sectioning. Parallel sections were cut parallel to the vertical axis. The used stereological software superimposed sine weighted test lines on fields of vision from the microscope.

Given the 0D of the test points no requirements were needed in order to create an isotropic test probe for estimation of volume fractions.

The purpose of the stereological design of the studies in this PhD thesis was to reduce the risk of introducing bias. However, a totally unbiased design is difficult to achieve and some degree of bias will most likely most introduced. The following section will discuss these potential biases.

Stereological bias

There are basically two sources of potential bias in microscopy, sampling bias and systematic bias. Sampling bias can be introduced if respective sections not are representative of the tissue in the histological specimen. Systematical bias can be divided into practical and theoretical bias. Practical bias can arise from the technical errors during the preparation process. A classic example of practical bias is when people estimate absolute values of volume densities in tissue that has shrink during preparation [144]. Theoretical bias arises when requirements for the test probes and objects of interest are not fulfilled. An example could be estimation of surface fractions on an anisotropic surface with the use of anisotropic test lines or the use of points to estimate number of objects.

The rest of this section will discuss potential sampling and systematical bias in the stereological design used in study I-III.

Sampling bias

The implant with its surrounding bone represents the region of interest (ROI). This ROI is embedded and sectioned. However, the histological sections cover only a fraction of the total ROI. It is important that the analyzed histological sections are representative of the ROI. If the tissue in the ROI were homogeneously distributed at every sampling level, then only one histological section would be necessary. This seems unlikely, and emphases the need for systematic uniform random sampling. In the present studies, the first 20-30 µm thick section from each specimen was uniform random cut from the center region of the implant. The subsequent three sections were systematically cut with a distance of 400 µm apart from each (Fig 12a). This implies that the sections cover approximately 1300 µm of the center region of the

implant. The sampling coverage could be enlarged by cutting the first section from a uniform random offset closer to the periphery of the implant and increasing the distance between the sections (Fig 12b). It seems reasonable to assume that an increased sampling coverage would produce more representative sections of the ROI.

The peri-implant zone of interest (PAI) is defined as concentric zone either 1000 µm (Study I and III) or 2000 µm (Study II) around the implant. On the histological sections the zone is outlined as a region beginning at the implant surface and ending 1000 µm (Study I and III) or 2000 µm (Study II) away from the implant surface. However, only a region on a histological sample cut directly through the center of the implant would coincide with the PAI. The fraction of the PAI covered by the region on the histological sample would decrease with increasing distance between the implant center and section off (Fig 13). The part not covered of the PAI is always the most peripheral zone of the PAI. It seems likely to assume that bone regenerative cells migrate from the periphery border of the PAI towards the implant surface, and that the different tissues within the PAI not are homogeneously distributed. Assuming an implant diameter of 6 mm, maximum section offset at 1.5 mm and a 2000 µm PAI, then the minimum fraction of the PAI covered by the region on the sections will be 93 %. It seems reasonable to assume that an acceptable coverage of the PAI can be obtained if the sections are cut within the central half of the implant. A high degree of coverage of the PAI was chosen at the cost of a reduced section sampling coverage, as discussed previously.

Practical bias

It is difficult to make thin histological section containing an implant. The average section thickness is assumed to be approximately 20-30µm. The relative large thickness will introduce a "shadow effect" when a cylindrical implant is cut parallel to its long axis (Fig. 14) [145]. When the section is cut in the periphery of the implant the "shadow effect" is increased. Increasing section thickness will also increase the "shadow effect". The "shadow effect" introduces a bias in the estimation of surface fraction, since the tissue in contact with the surface cannot be seen. Small layer of tissue in contact with the implant can thereby be overlooked. In order to reduce the impact of the "shadow effect" sections must be cut close to the implant center. Assuming an implant diameter of 6 mm, a section thickness of 30µm, and that the section are cut with a maximum offset of 1.5 mm from the implant center, then the maximum "shadow effect" can be calculated to by 17 µm. To put this in perspective, one can consider that the diameter of an osteoclast is around 50-100 µm. So, as long as the section sampling coverage is within 1.5 mm from the implant center the "shadow effect" will be acceptable.

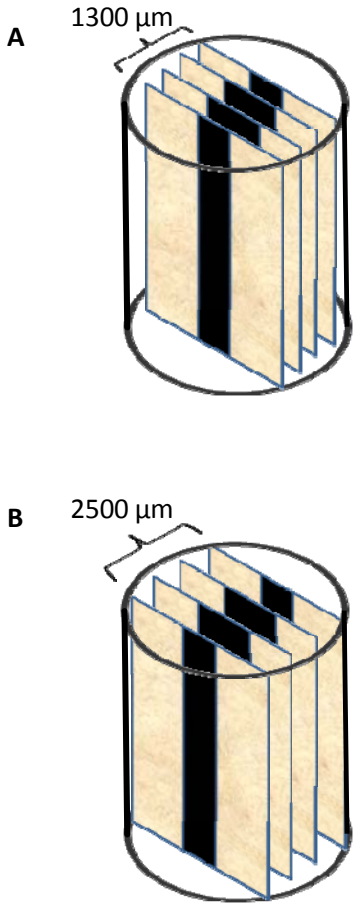


Fig. 12: The section sampling coverage can be increased by increasing the distance between the sections.

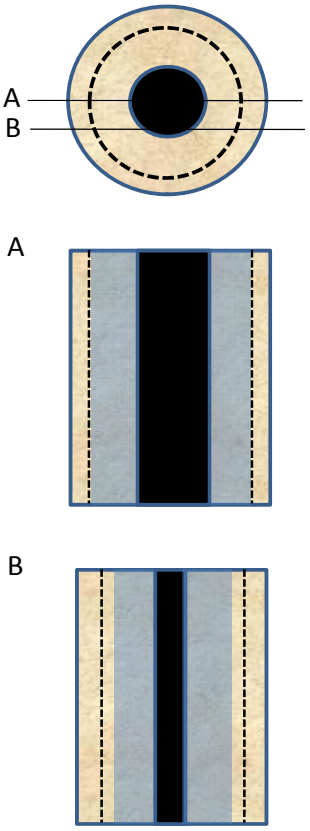


Fig. 13: Effect on section offset on coverage of peri-implant zone of interest (PAI). Top: transverse section of specimen with implant (black). Dotted line define outer border of PAI. Middle: section cut through center of implant (A). Bottom: section cut through the peripheral part of the implant (B). Shaded area indicates the sampling region covering a fixed area beginning at the implant surface. The sampling region does only cover the entire PAI at section A.

Another bias can arise from the relative thick sections, tissue over-projection. The phenomenon causes an overestimation of less transparent tissue compared to transparent tissue (Fig. 15). This implies that bone will be overestimated compared to marrow. Tissue over-projection can be reduced by only analyzing volume fractions in one focus plane. Furthermore, the penetration depth of light green into bone is 5-10 µm. Tissue over-projection can thereby further be reduced by only recording mineralized tissue stained green as bone. The bias contribution from tissue over-projection is considered small.

Estimation of valid surface fractions requires that the tissue originally in contact with the surface stays in contact during the preparation

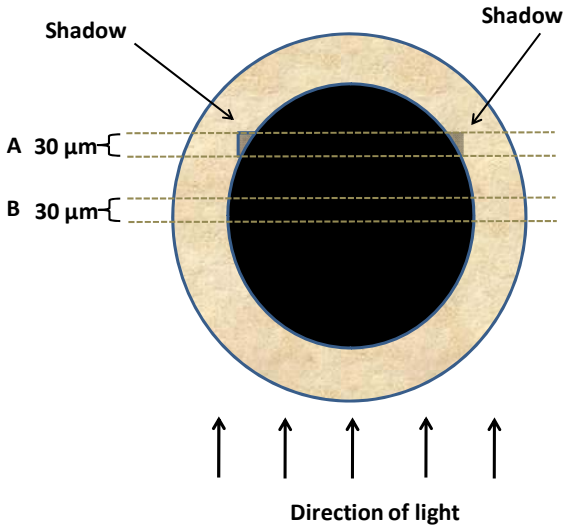


Fig. 14: Shadow effect. A 30 µm thick section (A) cut away from the center of the implant will cast a shadow on the tissue. Only a section (B) cut through the center of the implant will not cast a shadow.

process. Both the separation artifact and bone marrow can appear with similar morphology. This means that the fraction of bone marrow in contact with the implant can be overestimated at the cost of underestimation of bone in contact with the surface. It is difficult to estimate the impact of the separation artifact. Given the relative high bony surface coverage of many of the implant in the present studies, the impact is considered small.

Theoretical bias

It has previously been shown that the used implant model and section preparation technique can introduce "central section bias" [146]. The phenomenon introduces overrepresentation of tissue most likely to be present close to the implant surface and underrepresentation of tissue

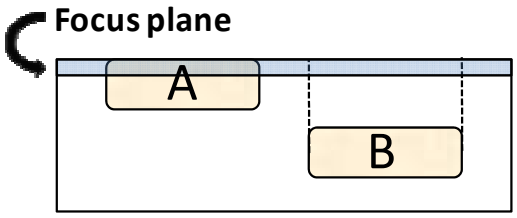


Fig. 15: Tissue over-projection. Bone deep (B) in the 20-30 µm thick section is projection into the focus plane. This over-projection can cause an overestimation of bone.

most likely to be presented far away far the surface. The phenomenon arises from the fact that points counted on close to the implant surface on section close to the vertical axis represent smaller volumes than points counted far away from the surface. This implies that the probability of a structure appearing in the central vertical section decreasing with the distance from the implant surface. It was estimated that the impact of the "central section bias" was acceptable [146].

Overall impact of bias

The previous discussion of stereological bias could indicate that used implant model and stereological design were too inaccurate. However, it is important to recognize that the presence of a bias does not mean that a study is highly inaccurate. Bias is almost inevitable and acceptable as long as their impact is small. However, the impact of a given bias is also relative to the study design. If the objective of a study is the estimate the true fraction of surface covered by bone, then a given bias impact would be relative high. If the objective of a study is the compare changes in fractions of surface covered by bone in a paired design, then a given bias impact would relative small. This mean that the paired design of the present studies help reduce the impact of the inevitable bias. It seems fair to conclude from the previous discussion that the impact of the present bias is small and acceptable.

Reproducibility

Measurements of intra-observer reproducibility were calculated from double measurements of randomly selected implant from all treatments groups. Reproducibility is expressed as coefficient of variance:

$$CV = \frac{\sqrt{½k \sum_1^k d^2}}{\bar{x}}$$

where,

CV = coefficient of variance

k = number of double estimates

d = difference between first and second double estimate

$\bar{x}$ = mean value of first and second estimate

Table 2: Reproducibility – Gap model

	New bone	Allograft	Marrow
Surface	18 %	0 %	1 %
Volume	4 %	6 %	1%

CV in percent

Table 3: Reproducibility – Compaction model

	Lamellar bone	Woven bone	Marrow	Total bone
Surface	48%	16%	9%	10%
Volume	2%	6%	0.2%	5%

CV in percent

The relative high CV values are caused by relative low fraction of the respective tissue. The CV values from the present studies are in accordance with other studies using the same model[146;147].

Statistical analysis

Statistical analysis was done using Intercooled Stata 9.0 (Stata Inc., College Station, TX, USA) on paired dataset from the treatment groups. All variables were normally distributed both before and after log transformation. Statistical analyses were done on ratios between paired data, which were not normally distributed. All variables were therefore log-transformed and Student's paired t-test was performed on absolute differences between normally distributed log-transformed paired data. An absolute difference between the logarithms of a pair of data equals the logarithm of the ratio within the pair [148]. Two tailed p-values below 0.05 were considered statistically significant. Results are presented as means of treatment groups or medians of relative differences between the paired data. The 95% confidence intervals for medians were obtained by back transformation of log-transformed data.

Summary of papers

Study I

Hypothesis

Local alendronate treatment will increase biomechanical implant fixation and osseointegration of Ti-coated implants inserted with bone compaction after 12 weeks in a canine model.

Surgery

All dogs were fully mobilized within 3 days of surgery. No dogs were excluded during the observation period. All bacterial cultures taken from the joint at time of euthanization were negative.

Biomechanical implant fixation

Alendronate caused an approximately twofold increase in all biomechanical parameters when comparing data from the alendronate group with their respective controls (Table 4).

Histology

Increased amount of bone was seen in contact with the implant surface of the alendronate implants compared to their controls. Around the alendronate implants a zone of relative high density bone was seen. Further away from the implants no qualitative difference was seen. No amounts of fibrous tissue were seen in any of the implants (Fig. 16).

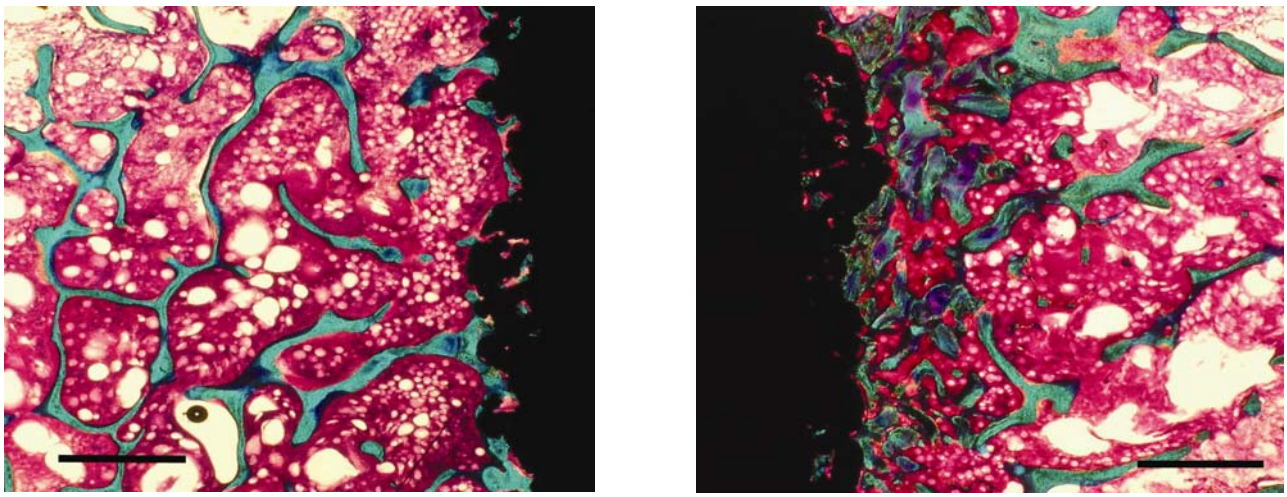


Fig. 16: Representative histological samples from the same animal. Control implant (left) and alendronate imlant (right). See text for description. Bar = 1.0 mm.

Table 4. Biomechanical Results - Study I

	Max Shear Strength, MPa	Max Shear Stiffness, MPa/mm	Total Energy Absorption, kJ/m^2
Control	3.03 (1.84;4.23)	12.3 (7.39;17.0)	0.95 (0.52;1.38)
Alendronate	6.65 (4.90;8.40)	23.6 (18.2;29.0)	1.69 (1.24;2.13)
Alendronate/Control	2.40 (1.78;3.25)*	2.23 (1.49;3.32)**	1.99 (1.37;2.90)***

Data are presented as mean for each treatment group (Control or Alendronate) or median for the relative paired increases (Alendronate/Control). 95%CI in parentheses. *p=0.0001, **=0.0015, ***p=0.0025

Table 5: Correlations between relative increases in histomorphometrical and biomechanical results – Study I

	Max. shear strength	Max Shear Stiffness	Total Energy Absorption
Bone-to-implant contact	0.34 (p = 0.007)	0.44 (p = 0.004)	0.029 (p =0.11)
Peri-implant bone density	0.81 (p < 0.0001)	0.076 (p = 0.0009)	0.27 (p = 0.12)

Data are presented as R-squared with p-values in parentheses. The R-squared should be interpreted as the fraction of the variance for increases in the respective biomechanical parameters that can be explained by the increases in the respective histomorphometrical parameters. E.g. 81% of the variance of the increase in max. shear strength can be explained by the increase in peri-implant bone density.

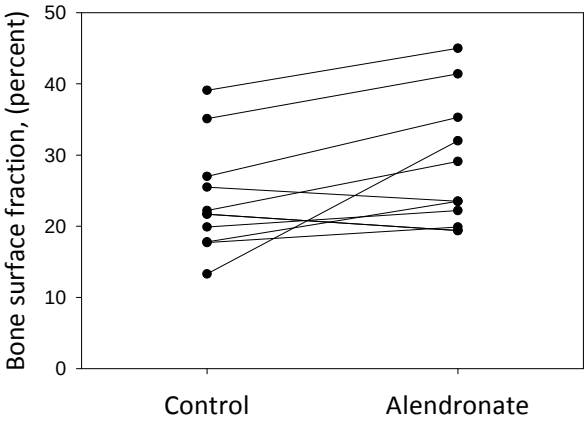


Fig. 17: Fraction of bone in contact with the implant surface. Paired data connected by line.

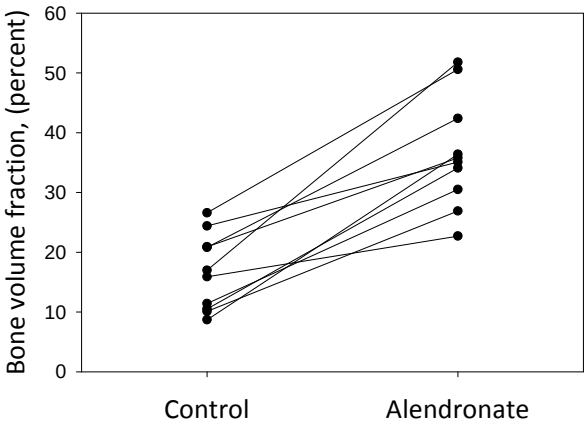


Fig. 18: Fraction of bone in a 1.0 mm zone around the implant. Paired data connected by line.

Histomorphometrical results

Local alendronate treatment caused a 23% median increase (95%CI: 1-49%, p = 0.04) in bone surface fraction and a 129 % median increase (95%CI: 77-197%, p <0.0001) in peri-implant bone volume fraction (Fig. 17 and 18). Correlations between biomechanical and histomorphometrical data can be seen in table 5.

Study II

Hypothesis

Impacting morselized allograft soaked in alendronate around Ti-coated implants will increase biomechanical implant fixation, increase new bone formation, and preserve the allograft after 4 and 12 weeks in a canine model.

Surgery

Two dogs were excluded due to a postoperative humerus shaft fracture and a clinically infected knee respectively. There were no signs of infection in the remaining dogs. All dogs were fully mobilized within 2 days of surgery.

Biomechanical implant fixation

Implants surrounded by alendronate soaked allograft from the 4- and 12-week observation period had significantly decreased biomechanical fixation compared with their respective controls (Table 6).

Histology

Increased amount of allograft were seen in the gap around the alendronate implants. The allograft seemed non-vital and with sparse amount of new bone formation in the outermost part of the gap. The allograft around the control implants from both observation periods seemed more remodeled and connected by new bone. The bone outside the gap seemed qualitatively unaffected (Fig. 19).

Histomorphometrical results

The formation of new bone in the gap was almost totally inhibited in both alendronate groups when compared with the respective controls (4 weeks, $p = 0.001$; 12 weeks, $p = 0.001$). No formation of new bone was observed on the surface of the implants from the alendronate groups (4 weeks, $p = 0.0006$; 12 weeks, $p = 0.0001$) (Fig. 20 and 21). Alendronate preserved the allograft in both observations periods, whereas the volume fraction of allograft around the control implants was reduced over time (4 weeks, $p < 0.0001$; 12 weeks, $p < 0.001$). Only a sparse amount of allograft was observed on the surface of the control implants compared with the alendronate implants (4 weeks, $p = 0.0026$; 12 weeks, $p = 0.0103$) (Fig. 22 and 23).

Table 6. Biomechanical Results - Study II

	Max Shear Strength, MPa	Max Shear Stiffness, MPa/mm	Total Energy Absorption, kJ/m^2
4 Weeks			
Control	3.7 (2.8;4.6)	15.0 (11.7;18.2)	0.91 (0.58;1.24)
Alendronate	0.08 (0.02;0.15)*	0.3 (0.0;0.4)*	0.03 (0.01;0.05)*
12 Weeks			
Control	6.7 (3.5;9.8)	24.7 (16.4;33.0)	1.7 (0.81;2.6)
Alendronate	0.22 (0.21;0.24)*	1.3 (0.5;2.1)*	0.08 (0.05;0.10)*

Data are presented as mean for each treatment group with 95%CI in parentheses. * < 0.0001 when comparing with controls from same observation period.

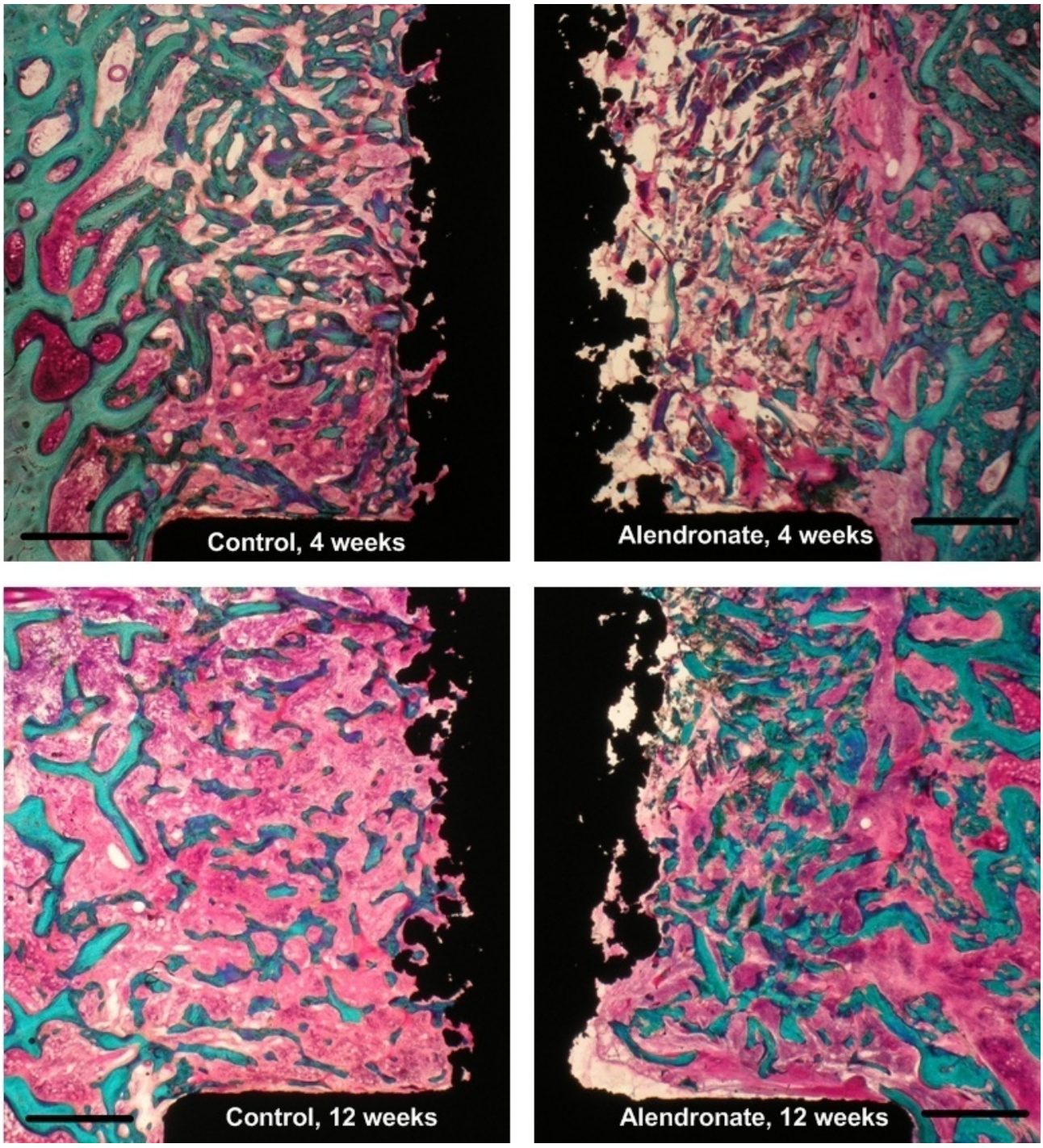


Fig. 19: Representative histological samples from the same animal. See text for description. Bar = 1.0 mm.

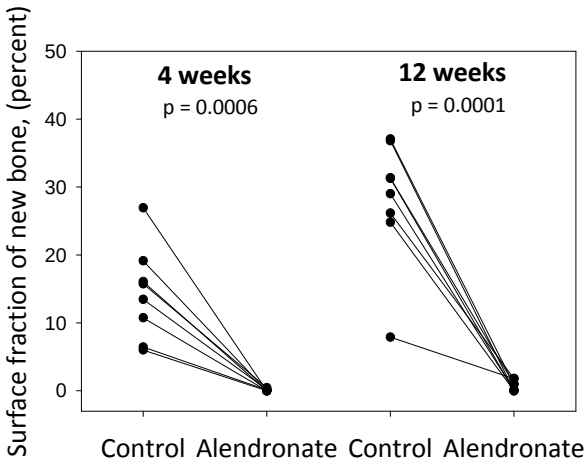


Fig. 20: Fraction of new bone in contact with the implant surface. Paired data connected by line.

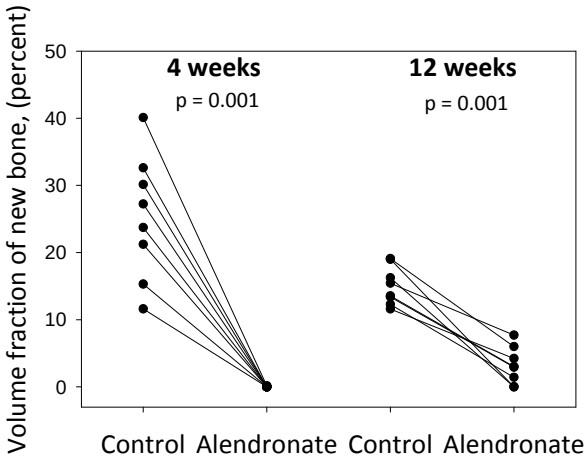


Fig. 21: Fraction of new bone in the gap around the implant. Paired data connected by line.

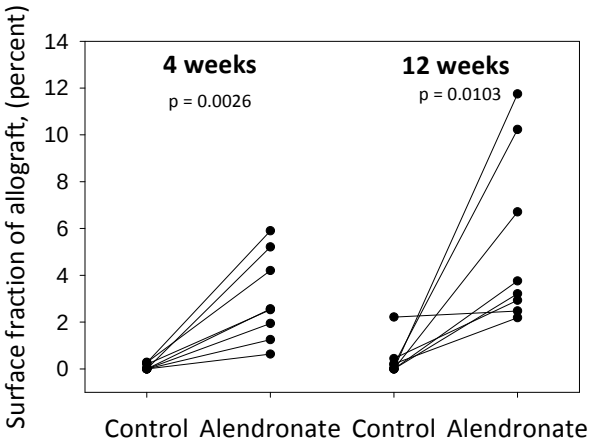


Fig. 22: Fraction of allograft in contact with the implant surface. Paired data connected by line.

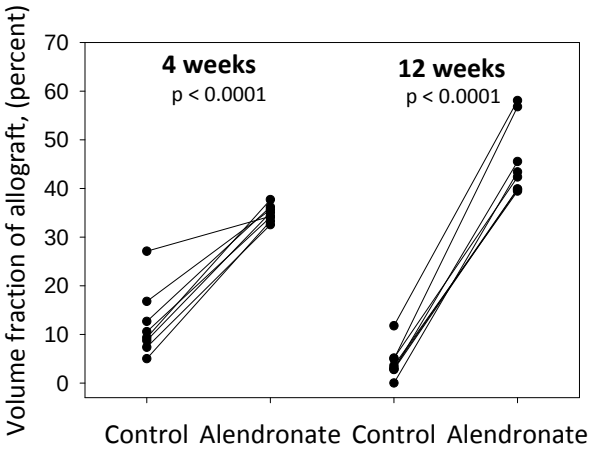


Fig. 23: Fraction of allograft in the gap around the implant. Paired data connected by line.

Study III

Hypothesis

Local alendronate treatment will increase biomechanical implant fixation and osseointegration of HA-coated implants inserted with bone compaction after 12 weeks in a canine model.

Surgery

All dogs were fully mobilized within 3 days of surgery. No dogs were excluded during the observation period. All bacterial cultures taken from the joint at time of euthanization were negative.

Biomechanical implant fixation

Alendronate resulted in an approximately 2.5-fold increase in maximum shear strength and maximum shear stiffness. No significant increase was found in total energy absorption (Table 7).

Histology

The most striking histological difference between the two groups was a 1 mm zone with relative dense cancellous bone around the implants from the alendronate group. Further away from the implant surface no histological difference in bone density was seen. The bone in the proximity of the implant from the alendronate consisted of lamellar bone chips and trabeculae covered with woven bone. The cancellous bone around the control implants seemed to more remodelled, since fewer lamellar bone chips were seen. No delaminating of the HA-coating was seen (Fig. 24).

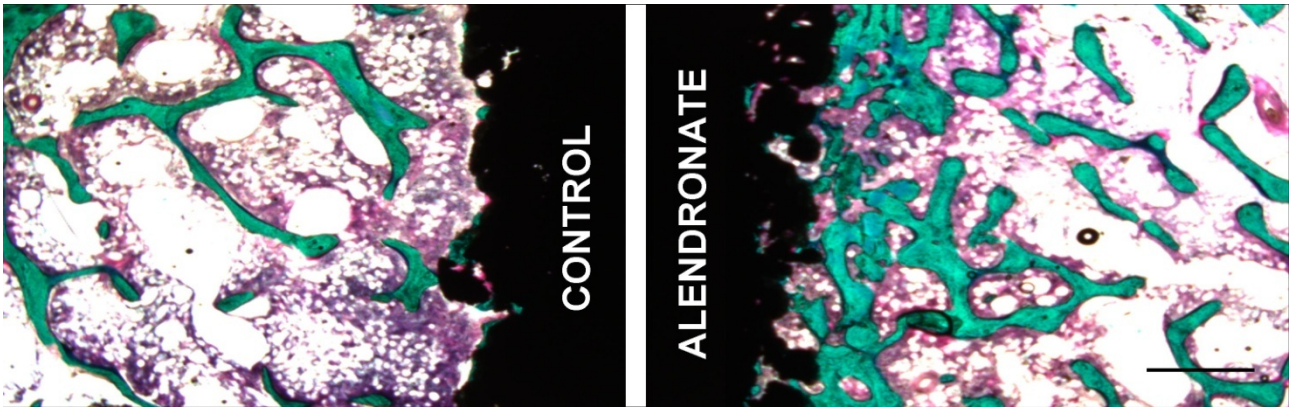


Fig. 24: Representative histological samples from the same animal. See text for description. Bar = 1.0 mm.

Table 7. Biomechanical Results - Study III

	Max Shear Strength, MPa	Max Shear Stiffness, MPa/mm	Total Energy Absorption, kJ/m^2
Control	1.59 (0.98;2.19)	10.0 (6.21;15.6)	0.30 (0.16;0.43)
Alendronate	2.91 (2.20;3.62)	25.2 (19.7;30.7)	0.45 (0.26;0.65)
Alendronate/Control	2.06 (1.44;2.95)*	2.72 (1.66;4.45)**	1.61 (0.90;2.86)***

Data are presented as mean for each treatment group (Control or Alendronate) or median for the relative paired increases(Alendronate/Control).95%CI in parentheses. *p=0.0014, **=0.0013, ***p=0.095

Table 8: Correlations between relative increases in histomorphometrical and biomechanical results – study III

	Max Shear Strength	Max Shear Stiffness	Total Energy Absorption
Bone surface fraction			
Woven bone	0.11 (p = 0.36)	0.01 (p = 0.75)	0.16 (p = 0.26)
Lamellar bone	0.57 (p = 0.012)	0.44 (p = 0.035)	0.45 (p = 0.033)
Bone volume fraction			
Woven bone	0.01 (p = 0.76)	0.01 (p = 0.82)	0.004 (p = 0.87)
Lamellar bone	0.53 (p = 0.016)	0.57 (p = 0.011)	0.26 (p = 0.14)

Data are presented as R-squared with p-values in parentheses. The R-squared should be interpreted as the fraction of the variance for increases in the respective biomechanical parameters that can be explained by the increases in the respective histomorphometrical parameters. E.g. 57% of the variance of the increase in max. shear strength can be explained by the increase in lamellar bone in contact with the implant surface.

Histomorphometrical results

The local alendronate treatment caused a 129% median increase (95%CI: 60-236%, p=0.0008) in peri-implant bone volume fraction. The increase was due to a 179% median increase (95CI%: 99-292%, p=0.0001) in woven bone volume fraction and a 127% median increase (95%CI: 43-262%, p=0.0031) in lamellar bone volume fraction (Fig. 25).

No difference in the amount of woven or lamellar bone in contact with the implant surface between the two groups was found (Fig. 25). Correlations between biomechanical and histomorphometrical data can be seen in table 8.

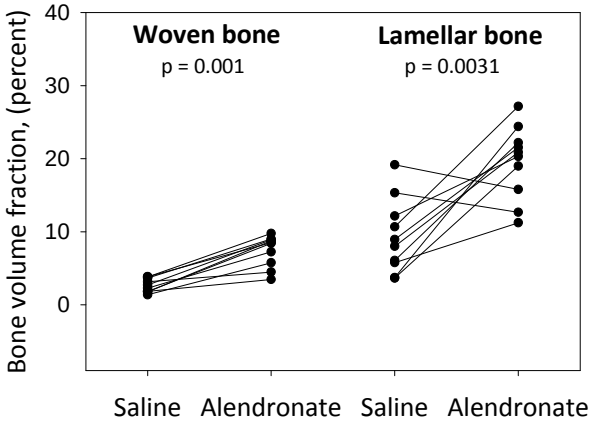


Fig. 25: Fraction of woven and lamellar bone in a 1.0 mm zone around the implant. Paired data connected by line.

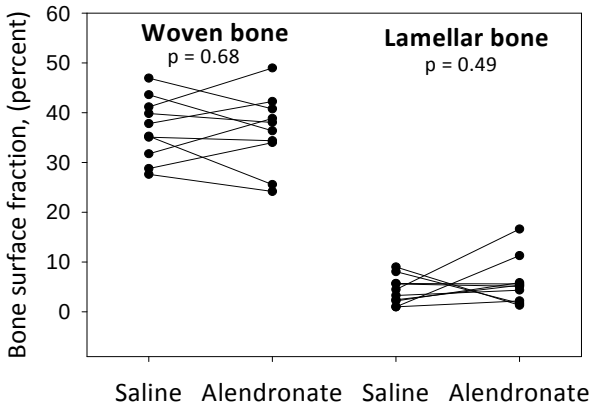


Fig. 26: Fraction of woven and lamellar bone in contact with the implant surface. Paired data connected by line.

Discussion

The specific aim of the present studies was to investigate the effect of local application of alendronate on early fixation and osseointegration of experimental implants in different settings. Locally applied alendronate was able to increase biomechanical fixation and osseointegration of implants inserted with the use of bone compaction (Study I and III). However, alendronate dramatically decreased biomechanical fixation and osseointegration of implants surrounded by morselized allograft (Study II).

The overall aim of this PhD thesis was to increase longevity of primary total hip arthroplasties (THA) and thereby reduce the risk of painful implant failure and costly revision. The used experimental models were designed to imitate a cementless THA inserted into cancellous bone. Extrapolation of the present results should be done in the context of the used experimental model and design.

As described in "Background", regeneration of bone around an implant is a dynamic process that occurs in several steps and different tempi. Study I and III compared implant fixation and osseointegration between control and intervention implant after 12 weeks. Study II had both a 4 and 12 weeks observation period. The observation periods represents cross-sections of the healing process. Discussion of treatment effects before and after these time-points can therefore only be speculative. The observation periods were chosen to represent early implant osseointegration. Previous studies have shown that differences in osseointegration can be detected at this stage [70;127;129]. Interpretation of the results from this PhD thesis should be done in the context of the observation periods.

Bisphosphonate can be administrated local or systemic. Local application of bisphosphonate makes it possible to obtain a high local concentration with minimum systemic effect. Systemic application makes it possible to administrate bisphosphonate both pre-, per- and post-operative. Local bisphosphonate treatment can only be given per-operative, but it can be targeted to areas with minimum blood supply as opposed to systemic treatment. An advantage of local application in the context of experimental research is the possibility to make a paired design with a relative low number of animal and eliminated inter-individual variation. Bisphosphonate was applied locally in the present studies. This means that bisphosphonate could potentially influence the bone around the control implant and diminish a potential treatment effect. However, results from the control implants in both the allograft and compaction studies were comparable to results obtained in other studies using the same model, but without bisphosphonate treatment [70;130;149]. This suggests that locally applied bisphosphonate did not have an effect on the control implants. Additionally, even if the alendronate might have exerted a systemic effect, these studies are still able to detect significant difference in treatment effect.

Bisphosphonate and compaction

Study I and III investigated the effect of rinsing a bone cavity with alendronate before compaction the surrounding bone and subsequently inserting a Ti- or HA- coated implant. 5 mL saline containing 10 mg alendronate was used to rinse the bone cavity. The used amount and concentration of bisphosphonate was based on a previous study using a similar model and the literature [121;133]. Only speculations about the effects of other concentrations and amounts of alendronate can be drawn for these studies.

When bisphosphonate is locally added to cancellous bone most of it will adsorb to the bone surface while a small amount will stay unbound in solution between the trabeculae. A too high concentration of the unbound bisphosphonate may not only inhibit the osteoclasts but also the

osteoblasts, and thus new bone formation [96]. It is important that the concentration of unbound free bisphosphonate is below the toxic level. The omission of irrigating a bone cavity after soaking it in bisphosphonate could therefore be potential deleterious. In study I and III no irrigation of the bone cavity was done after soaking it in alendronate. However, the studies were still able to demonstrate that local alendronate treatment could increase biomechanical fixation and implant osseointegration. It could be that bleeding from the marrow cavity or suction applied to remove excess bisphosphonate after the soaking period was able to reduce the amount of unbound alendronate. An efficient and safe way to remove unbound potential toxic bisphosphonate could be with irrigation of the drill hole after soaking the bone in bisphosphonate.

One of the specific aims of this thesis was to increase to biomechanical implant fixation. Study I and III demonstrated that local alendronate treatment was able to significant increase the biomechanical fixation of both Ti- and HA- coated implants inserted with the use of bone compaction. This increase can be partly explained by the increases in the amount of bone in contact with and around the implants. This is supported by correlations between increases in histomorphometrical and biomechanical results (Table 5 and 8). It seems from these correlations that increases in lamellar bone are relative more important than increases in woven when explaining the increases in biomechanical fixation. Note however, it is dangerous to extrapolate these results. A high bone density is not always correlated to a strong biomechanical implant fixation. Study II is an example of this. The relative strong correlation between increases in lamellar bone and increases in biomechanical implant fixation might partly be explained by the spring back effect of compacted bone [76]. The elastic properties of the compacted lamellar bone ensure that the implant is placed in initial extreme-fit. Local bisphosphonate treatment has the ability to preserve this bone while new bone is formed.

Local alendronate treatment was able to significant increase both the amount of bone around the implant (Study I and III) and on the surface (Study I). This is in accordance with others studies [114;116]. Implants in study I and III were inserted with use of bone compaction. The bone compaction technique creates a zone of compacted autograft around the implants [70]. A preservation of this autograft created *in situ* by alendronate could explain the relative increase in lamellar bone. Furthermore, a preservation of the compacted bone would result in a larger surface increasing new bone formation through the process of osteoconduction. This could explain the relative increase in woven bone seen around the implants from the alendronate group. Another explanation for the increased amount of new bone could be that the anti-resorptive effect of alendronate prolongs the remodelling of woven bone. This is in accordance with others studies [122;123].

No difference was found in the amount of bone in contact with the HA-coated implant surface between the groups in study III. Other studies have shown a positive effect of bisphosphonate on bone in contact with a HA-coated implant [116;150]. A threshold could exit, beyond which the bone-to-implant contact is extremely difficult to enhance. The implants in study III are placed in extreme press-fit due to the spring-back effect of the compacted bone, and thereby in contact with a relative high amount of bone at time zero [76]. Furthermore, the HA-coating on the implants in this study is known to have osteoconductive properties [151]. It could be that the combined effect of the bone compaction technique and HA-coating leaves little room for improvement by alendronate in this model.

Bisphosphonate and allograft

Study II investigated the effect of soaking morselized allograft in alendronate before impacting it around a Ti-coated implant.

Bisphosphonates have previously been shown in experimental studies to prevent graft

resorption while allowing new bone to be formed [121-123]. An expected outcome of this study would be an increased amount of new bone and preserved allograft in the alendronate group, both leading to increased biomechanical implant fixation and implant osseointegration. Soaking morselized allograft in alendronate resulted in preservation of allograft, but virtually no formation of new bone and a dramatic decrease in biomechanical implant fixation. These findings were unexpected.

Fixation of cementless implants is dependent on osseointegration [22]. To obtain secure fixation of implants surrounded by morselized allograft, the allograft must be incorporated with new bone. The formation of new bone within the allograft can take place as intramembranous ossification on the allograft surface or as a creeping substitution [55]. It has been shown that impaction of allograft decreases its osteoconductivity [57]. One explanation could be the relative high density of allograft grains may not provide space for ingrowth of tissue or blood vessels. This may imply formation of new bone within impacted allograft is primarily dependent on creeping substitution which, in turn, is dependent on bone resorption. Because alendronate inhibits bone resorption, it may thereby inhibit ingrowth of new bone into the allograft in the process of creeping substitution. This might explain why we observed a decreased amount of new bone around the implants surrounded by impacted morselized allograft that had been soaked in alendronate. Furthermore, the poor biomechanical fixation of the alendronate implants could be explained by the impaired osseointegration.

Another likely explanation for the impaired bone formation could be the selected dose of alendronate was too high. In this study, we soaked the allograft in 10 mg dissolved in 5 mL saline. This dose was based on the encouraging results from study I where the same dose was used. Local treatment of allograft has previous resulted in a decreased bone resorption and increased bone formation [121]. However, in this study, unbound bisphosphonate was rinsed away with saline. In study II the allograft was not rinsed after it had been soaked in alendronate. The omission of rinsing together with the high concentration of alendronate could imply that the excess bisphosphonate exerts a local toxic effect. It could be that a lower concentration of bisphosphonate or rinsing the unbound bisphosphonate away would have resulted in an increased implant fixation.

The results from study II are in accordance with a previous study using the same implant model [149]. In this study it was found the local treatment with the bisphosphonate pamidronate blocked allograft resorption and new bone formation. The results were explained with the applied dose and application method. Given the similarities between this study[149] and study II, it is impossible to conclude which of the above explanations is most plausible. It can, however, be concluded that the observed effect most likely is independent of the type of bisphosphonate.

The discrepancy between the results from study II and the encouraging results from the literature indicate that a therapeutic window exits [121-123]. It also emphasizes the importance of preclinical testing, since bisphosphonates can inhibit new bone formation and potentially impair biomechanical implant fixation. The unexpected results from study II warrant a further preclinical investigated.

Results in a clinical context

The results from study I and III are promising. They indicate that there may be a clinical advantage in the use of topical bisphosphonate in total joint replacements inserted with the use of bone compaction. A potential application could be the irrigation of exposed surfaces of cancellous bone with a bisphosphonate solution prior to bone compaction and subsequent implantation of the prosthesis component. This could protect the patient's own bone from early resorption pending remodelling and strengthening of the newly formed peri-implateric bone. To prevent potentially adverse effects of unbound

bisphosphonate, it is probably advisable to irrigate with saline after irrigation with the bisphosphonate solution.

One concerns regarding the use of bisphosphonate as adjuvant in implant fixation is the effect of decreasing bone turnover. An impaired bone turnover might lead to increased bone fragility and accumulation of micro fractures, meaning reduced implant fixation [105]. This emphasizes the need to study the long-term effects of bisphosphonate on implant fixation before implementing the treatment in the clinical setting.

Conclusion

The results from the present studies are diverting. Two of the studies demonstrate that local alendronate treatment can increase implant fixation and osseointegration of experimental implants inserted with the use of bone compaction. One of the studies demonstrates that local alendronate treatment has the ability to block formation of new bone in impacted, morselized allograft and to decrease biomechanical implant fixation. The present studies are limited by the selected dose of alendronate, application method and observation periods, and warrants further preclinical investigation. The results of future investigation will show whether bisphosphonates have a place as adjutants in total joint replacements.

Suggestion for future research

The results form study I and III are promising. It was shown that the early implant fixation and osseointegration could be enhanced with the use of local alendronate treatment on implant inserted with bone compaction. However, given the decreased bone turn-over as a result of bisphosphonate treatment, it is difficult to extrapolate the results to longer follow-up periods. It would be of interest to repeat study I and III with a longer follow-up.

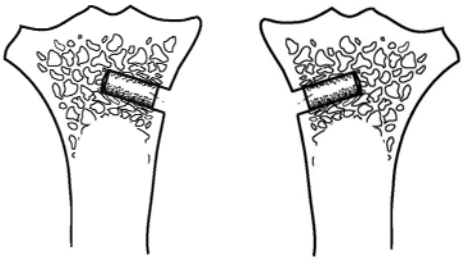
The implants in the present studies were all non-weight-bearing. The transfer of weight through the bone-implant will result in increased stress in the bone around the implant. Furthermore, accumulation of micro fractures due to bisphosphonate treatment could lead to increased concentration of stress-centers in the bone resulting in more micro fractures [105]. This vicious circle could lead to implant failure. It would be of interest to investigate the effect weight-bearing on implant fixation of implants inserted into bisphosphonate treated bone.

The results from study II were unexpected. It was expected that soaking morselized allograft in alendronate and subsequently impacting it around an experimental implant would reduce graft resorption, increase new bone formation and enhance biomechanical implant fixation. Alendronate blocked graft resorption and new bone formation, and decreased biomechanical implant fixation. It is imperative to investigate whether the observed results are due to the used dose and omission of rinsing unbound potential toxic alendronate away. It would be of interest to perform of dose-response study.

Thesis at a glance

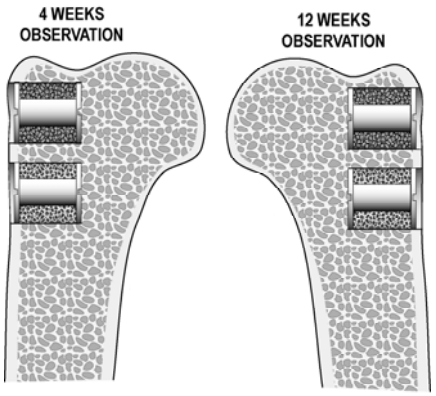
Paper I

Hypothesis: Local bisphosphonate treatment can increase fixation of implants inserted with the use of bone compaction.
Design: Implants were inserted into tibia using bone compaction. Bone treated locally with alendronate or saline.
Implant coating: Titanium.
Observation time: 12 weeks.
Results: Increased biomechanical implant fixation and osseointegration.



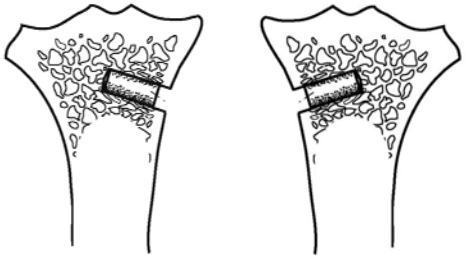
Paper II

Hypothesis: Impacting morselized allograft soaked in bisphosphonate around implants can increase fixation of implants, and reduce allograft resorption.
Design: Implants surrounded by impacted morselized allograft either soaked in alendronate or saline were inserted into humerus.
Implant coating: Titanium.
Observation period: 4 and 12 weeks.
Results: Alendronate reduced allograft resorption, but blocked new bone formation and reduced biomechanical implant fixation.



Paper III

Hypothesis: Local bisphosphonate treatment can increase fixation of implants inserted with the use of bone compaction.
Design: Implants were inserted into tibia using bone compaction. Bone treated locally with alendronate or saline.
Implant coating: Hydroxy-apatite.
Observation time: 12 weeks.
Results: Increased biomechanical implant fixation and osseointegration.



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   Martin Lind, January 1996

2. Gene delivery to articular cartilage.
   Michael Ulrich-Vinther, September 2002

3. The influence of hydroxyapatite coating on the peri-implant migration of polyethylene particles.
   Ole Rahbek, October 2002

4. Surgical technique's influence on femoral fracture risk and implant fixation. Compaction versus conventional bone removing techniques.
   Søren Kold, January 2003

5. Stimulation and substitution of bone allograft around non-cemented implants.
   Thomas Bo Jensen, October 2003

6. The influence of RGD peptide surface modification on the fixation of orthopaedic implants.
   Brian Elmengaard, December 2004

7. Reaming procedure and migration of the uncemented acetabular component in total hip replacement.
   Thomas Baad-Hansen, February 2007

8. Biological response to wear debris after total hip arthroplasty using different bearing materials.
   Marianne Nygaard, June 2005

9. DEXA-scanning in description of bone remodeling and osteolysis around cementless acetabular cups.
   Mogens Berg Laursen, November 2005

10. Studies based on the Danish Hip Arthroplastry Registry.
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11. On the longevity of cemented hip prosthesis and the influence on implant design.
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12. Combination of TGF-β1 and IGF-1 in a biodegradable coating. The effect on implant fixation and osseointegration and designing a new in vivo model for testing the osteogenic effect of micro-structures in vivo.
    Anders Lamberg, June 2007

13. Evaluation of Bernese periacetabular osteotomy; Prospective studies examining projected load-bearing area, bone density, cartilage thickness and migration.
    Inger Mechlenburg, August 2007
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14. Rehabilitation of patients aged over 65 years after total hip replacement - based on patients' health status.
    Britta Hørdam, February 2008

15. Efficacy, effectiveness, and efficiency of accelerated perioperative care and rehabilitation intervention after hip and knee arthroplasty.
    Kristian Larsen, May 2008

16. Rehabilitation outcome after total hip replacement; prospective randomized studies evaluating two different postoperative regimes and two different types of implants.
    Mette Krintel Petersen, June 2008

17. CoCrMo alloy, in vitro and in vivo studies.
    Stig Storgaard Jakobsen, June 2008

18. Adjuvant therapies of bone graft around non-cemented experimental orthopaedic implants. Stereological methods and experiments in dogs
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4. Adult hip dysplasia and osteoarthritis. Studies in radiology and clinical epidemiology.
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5. Gene therapy methods in bone and joint disorders. Evaluation of the adeno-associated virus vector in experimental models of articular cartilage disorders, periprosthetic osteolysis and bone healing.
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