Improving implant fixation with bisphosphonates

Doctoral dissertation

Thomas Jakobsen, MD, PhD

Health
Aarhus University
2019

Denne afhandling er d.d. sammen med de anførte, tidligere offentliggjorte artikler af fakultetet Health ved Aarhus Universitet antaget til forsvar for den medicinske doktorgrad.

Aarhus Universitet, 14. februar 2019

Lars Bo Nielsen
Dekan

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List of papers

This doctoral thesis is based on the following 11 papers, referred to in the text by Roman numerals (**I -XI**):

I. **Jakobsen T**, Kold S, Bechtold JE, Elmengaard B, Søballe K. Effect of topical alendronate treatment on fixation of implants inserted with bone compaction. *Clin Orthop Relat Res* 2006;444:229–34.

II. **Jakobsen T**, Kold S, Bechtold JE, Elmengaard B, Søballe K. Local alendronate increases fixation of implants inserted with bone compaction: 12-week canine study. *J Orthop Res* 2007;25:432–41.

III. **Jakobsen T**, Baas J, Bechtold JE, Elmengaard B, Søballe K. Soaking morselized allograft in bisphosphonate can impair implant fixation. *Clin Orthop Relat Res* 2007;463:195–201.

IV. **Jakobsen T**, Baas J, Kold S, Bechtold JE, Elmengaard B, Søballe K. Local bisphosphonate treatment increases fixation of hydroxyapatite-coated implants inserted with bone compaction. *J Orthop Res* 2009;27:189–94.

V. **Jakobsen T**, Baas J, Bechtold JE, Elmengaard B, Søballe K. The effect of soaking allograft in bisphosphonate: a pilot dose-response study. *Clin Orthop Relat Res* 2010;468:867–74.

VI. **Jakobsen T**, Baas J, Bechtold JE, Elmengaard B, Soballe K. The Effect on Implant Fixation of Soaking Tricalcium Phosphate Granules in Bisphosphonate. *Open Orthop J* 2012;6:371–5.

VII. **Jakobsen T**, Kold S, Baas J, Søballe K, Rahbek O. Sheep Hip Arthroplasty Model of Failed Implant Osseointegration. Open *Orthop J* 2015;9:525–9.

VIII. **Jakobsen T**, Bechtold JE, Søballe K, Jensen T, Greiner S, Vestermark MT, Baas J. Local delivery of zoledronate from a poly (D,L-lactide)-coating increases fixation of press-fit implants. *J Orthop Res* 2016;34(1):65-71.

IX. **Jakobsen T**, Bechtold JE, Søballe K, Jensen T, Vestermark MT, Baas J. Local delivery of zoledronate from a poly (D,L-lactide)-coating increases fixation of hydroxy-coated

implants. *J Orthop Res* 2017;35:974-9.

X. Baas J, Vestermark MT, Jensen T, Bechtold JE, Soballe K, **Jakobsen T**. Topical Bisphosphonate Augments Fixation of Bone-grafted Hydroxyapatite-coated Implants, BMP-2 causes Resorption-based decrease in Bone. *Bone* 2017;97:76-82.

XI. **Jakobsen T**, Kold S, Shiguetomi-Medina, Baas J, Soballe K, Rahbek O. Topical Bisphosphonate decreases Micromotion induced Bone Resorption in a Sheep Arthroplasty Model. *BMC Musculoskelet Disord* 2017;18:441.

Papers II, III, and IV were included in my PhD thesis, *The Influence of Local Bisphosphonate Treatment on Implant Fixation*, Aarhus University, 2008.

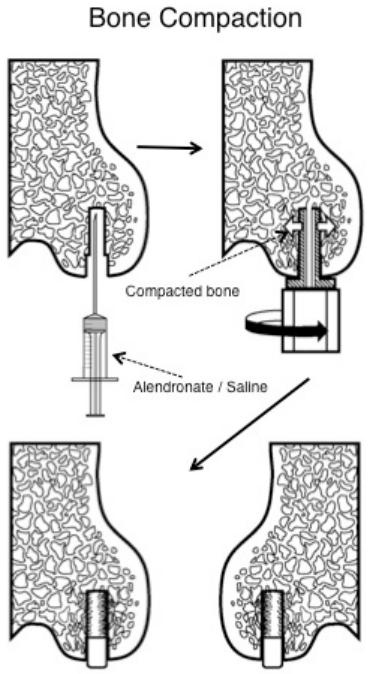
Contents

Thesis at a glance

Summary of papers

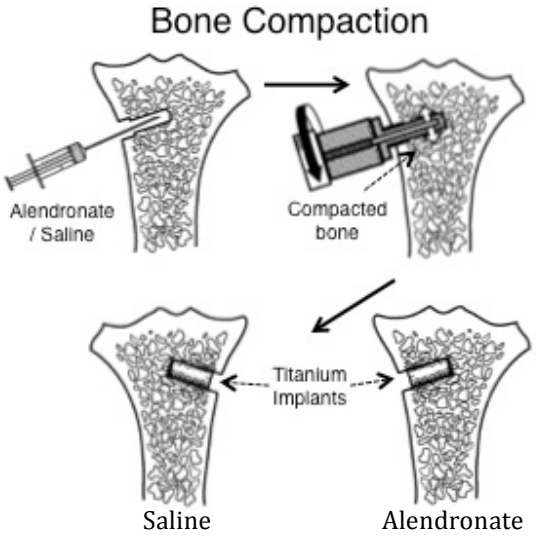
Paper I

Hypothesis: Local bisphosphonate treatment can increase fixation of weight-bearing implants inserted with the use of bone compaction.
Implant model: Weight-bearing bone compaction. Canine.
Design: Implants were inserted using bone compaction. Bone bed treated locally with alendronate or saline
Implant coating: Plasma sprayed porous titanium.
Observation time: 4 weeks.
Results: Alendronate increased the amount of lamellar bone. No difference in the amount of new bone. No improvement in biomechanical fixation.



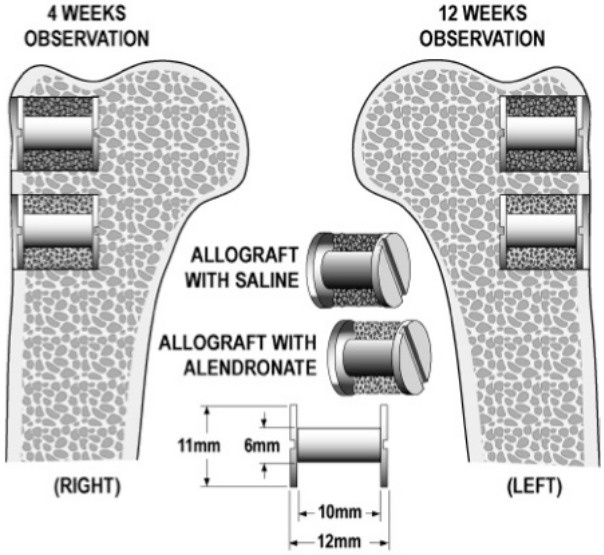
Paper II

Hypothesis: Local bisphosphonate treatment can increase fixation of unloaded implants inserted with the use of bone compaction.
Implant model: Bone compaction. Canine.
Design: Implants were using bone compaction. Bone treated locally with alendronate or saline.
Implant coating: Plasma sprayed porous titanium.
Observation time: 12 weeks.
Results: Increased biomechanical implant fixation and osseointegration.



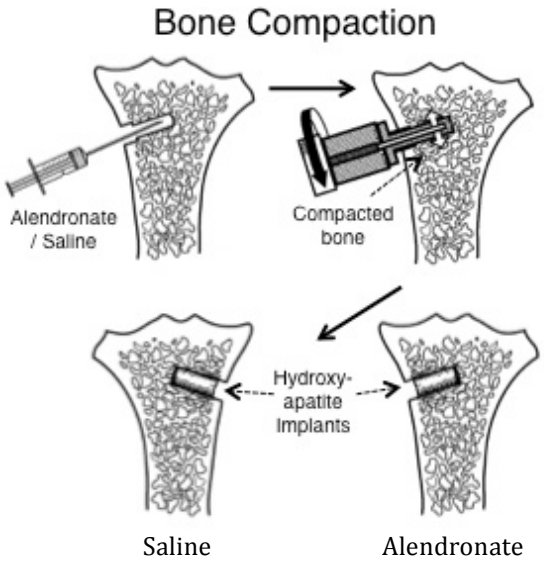
Paper III

Hypothesis: Impacting morselized allograft soaked in bisphosphonate around implants can increase fixation of implants, and reduce allograft resorption.
Implant model: 2.5 mm gap model. Canine.
Design: Paired design with 2 implants in each humerus. One pair inserted at time zero. Second pair inserted in contralateral humerus 8 weeks later. Implants were surrounded by impacted morselized allograft either soaked in alendronate or saline.
Implant coating: Plasma sprayed porous titanium.
Observation period: 4 and 12 weeks.
Results: Alendronate reduced allograft resorption, but blocked new bone formation and reduced biomechanical implant fixation.



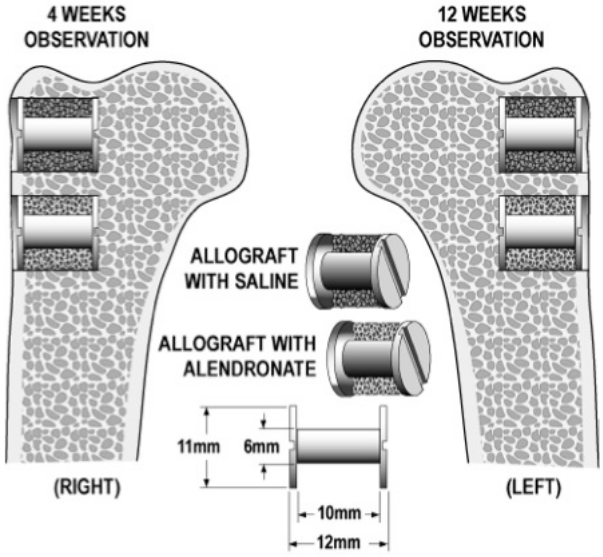
Paper IV

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



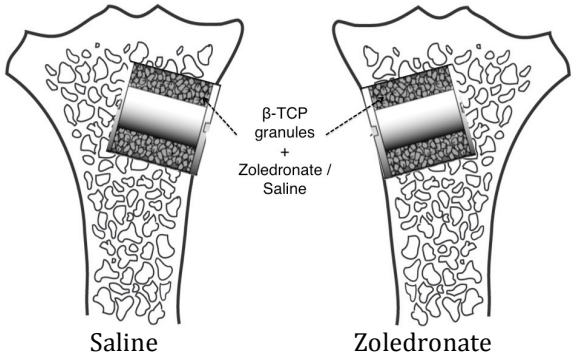
Paper V

Hypothesis: Impacting morselized allograft around an implant after it has been soaked in bisphosphonate can preserve allograft; increase implant fixation and osseointegration in a dose-dependent manner.
Implant model: 2.5 mm gap model. Canine.
Design: Implants were surrounded by impacted morselized allograft either soaked in saline or low-, middle- or high-dose of zoledronate and subsequently rinsed
Implant coating: Plasma sprayed porous titanium.
Observation period: 4 weeks.
Results: Biomechanical implant fixation and new bone formation was affected in a dose-dependent manner with the lowest dose of zoledronate given the best results. Increasing concentrations of zoledronate resulted in increased preservation of allograft.



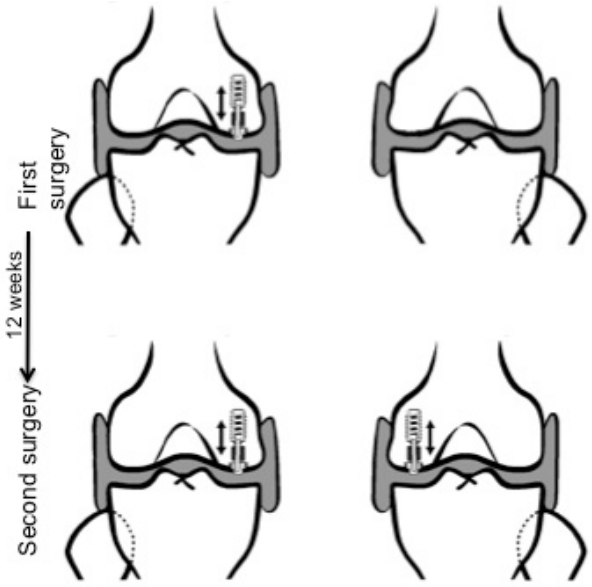
Paper VI

Hypothesis: β-TCP granules soaked in zoledronate and impacted around an implant will increase biomechanical implant fixation, enhance new bone formation and preserve β-TCP granules.
Implant model: 2.5 mm gap model. Canine.
Design: Implants were surrounded by impacted β-TCP granules soaked in zoledronate or saline.
Implant coating: Sintering bead porous titanium.
Observation period: 12 weeks.
Results: Zoledronate increased one of three biomechanical parameters, but did not affect amount of new bone or β-TCP granules.



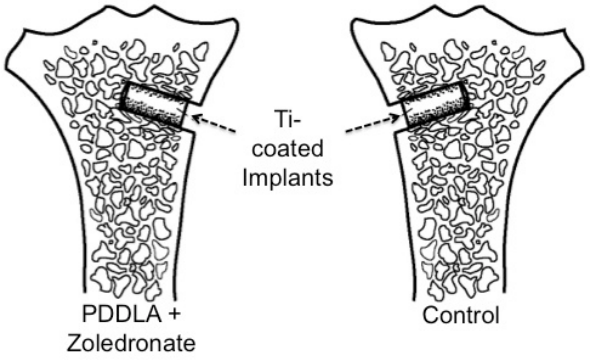
Paper VII

Hypothesis: Implant micromotion will prevent osseointegration, increase peri-implant bone resorption and induce formation of fibrous tissue.
Implant model: Exact-fit micromotion implant. Sheep.
Design: One micromotion implant observed for 12 weeks. Time zero implant inserted postmortem.
Implant coating: PMMA.
Observation period: zero and 12 weeks.
Results: Micromotion induced bone resorption and formation of a fibrous membrane.



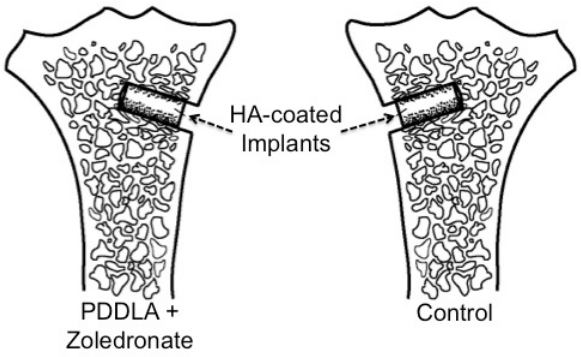
Paper VIII

Hypothesis: Zoledronate eluted from a PDLLA coating will increase implant fixation and osseointegration.
Implant model: Exact–fit implant. Canine.
Design: One implant coated with PDLLA containing zoledronate was inserted into the proximal part of tibia. Uncoated implant was inserted in the contralateral side.
Implant coating: Sintering bead porous titanium.
Observation period: 12 weeks.
Results: Zoledronate increased biomechanical implant fixation and amount of bone around the implants. No effect was observed on implant osseointegration.



Paper IX

Hypothesis: Zoledronate eluted from a PDLLA coating will increase implant fixation and osseointegration.
Implant model: Exact–fit implant. Canine.
Design: One implant coated with PDLLA containing zoledronate was inserted into the proximal part of tibia. Uncoated implant was inserted in the contralateral side.
Implant coating: Sintering bead porous hydroxy-apatite.
Observation period: 12 weeks.
Results: Zoledronate increased biomechanical implant fixation and amount of bone around the implants. No effect was observed on implant osseointegration.



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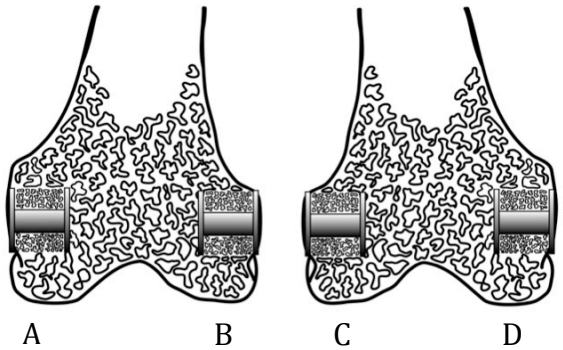
Paper X

Hypothesis: Combination of BMP-2 and zoledronate will increase implant fixation and osseointegration of implants surrounded by morselized allograft.
Implant model: 2.5 mm gap. Canine.
Design: Implant surrounded by allograft. Treatment groups: A) control, B) BMP-2, C) zoledronate, D) BMP-2 and zoledronate.
Implant coating: Sintering bead porous hydroxy-apatite.
Observation period: 4 weeks.
Results: Zoledronate preserved allograft, increased implant fixation and osseointegration. BMP-2 induced allograft resorption, increased new bone formation and impaired implant fixation. Zoledronate did not counteract the increased allograft resorption induced by BMP-2. BMP-2 and zoledronate in combination decreased implant fixation.



Paper XI

Hypothesis: Zoledronate can reduce bone resorption and fibrous tissue formation around implants subjected to micromotion.

Implant model: Exact-fit micromotion implant. Sheep.

Design: One micromotion implant in each knee. Bone bed soaked in zoledronate or saline.

Implant coating: PMMA.

Observation period: 12 weeks.

Results: Zoledronate reduced bone resorption and fibrous tissue formation, but did not prevent it.

What this thesis adds to literature

Longevity of a joint prosthesis is dependent on secure initial implant fixation[1–4]. One way to improve longevity is to improve the initial fixation.

Common for all joint prostheses is that they transfer load from the implant to the bone. This load is transferred through the interface between the implant surface and surrounding bone or cement. From the interface, the load is transmitted through the peri-implant bone bed and further into the load-bearing part of the bone. Allograft material can be used to optimize the quality of the bone bed in situation with bone loss.

Failure of implant fixation can occur at all links in this "chain of implant fixation". **Study I-XI** in this thesis investigated how to improve the different links in this chain (Fig. 1). Common for all studies was that they investigated the effect of local bisphosphonate treatment on implant fixation. Bisphosphonates are drugs that inhibit bone resorption.

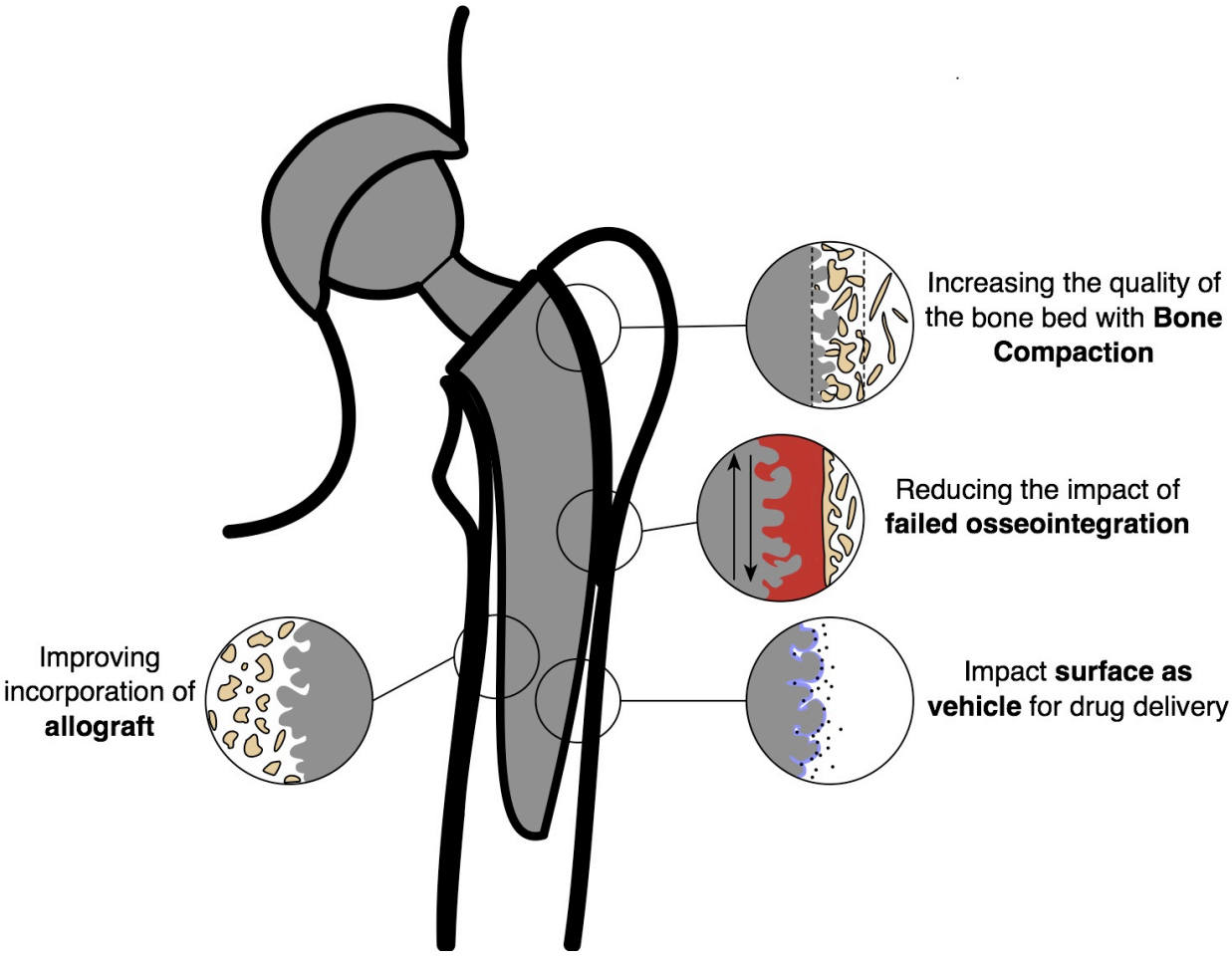


Figure 1. "Chain of implant fixation". Targets to improve investigated in this thesis.

Major findings

Bone compaction (Study I-II and IV)
The bone compaction technique enhances the quality of the bone bed by creating a dense zone of compacted bone autograft *in situ*. Furthermore, it places the implant in extreme press fit due to the *spring back effect* of the bone bed.

Local treatment with bisphosphonates:

- can further increase the mechanical implant fixation*
- preserves the zone of autograft
- increases formation of new bone*

Bone graft (Study III, V-VI and X)
Morselized bone allograft can be used to enhance the quality of the bone bed in situations with compromised bone stock. Furthermore, it adds primary mechanical stability to the implant. An alternative to bone allograft is synthetic bone graft substitutes such as TCP. BMP-2 can accelerate allograft resorption and increase new bone formation.

Local treatment with bisphosphonates:

- can increase mechanical implant fixation
- preserves the allograft
- increases new bone formation
- displays a dose-response relationship with respect to new bone formation
- can impair new bone formation*
- is not able to counteract the increased allograft resorption induced by BMP-2

Implant surface (Study VIII-IX)
Osseointegration can be enhanced by local delivery of drugs to the implant-bone interface. The implant surface can be used as a vehicle for this drug delivery.

Local delivery of bisphosphonate from a PDLLA surface coating:

- can increase mechanical implant fixation
- can increase formation of new bone and preservation of old bone around implants
- can't increase implant osseointegration

Failed osseointegration (Study VII and XI)
Stable primary mechanical implant fixation is necessary for secondary biological implant osseointegration. Unstable primary implant conditions stimulate bone resorption. This bone resorption might be reduced with local bisphosphonate treatment.

Study VII and XI showed that:

- it is possible to create a model of failed primary implant fixation
- local bisphosphonate treatment can reduce micromotion induced bone resorption, but not prevent it.

* Findings from studies included in my Ph.D. thesis.

Introduction to implant fixation

The first total hip arthroplasty

The first attempt to perform a total hip arthroplasty (THA) was carried out in 1891 in Berlin, Germany, by Professor Themistocles Glück (1853-1952) [5]. His implant was made of ivory and used to replace a femoral head that have been destroyed by tuberculosis.

In 1925, Marius Smith-Petersen (1886-1953) created the first mold arthroplasty. His implant was made of glass and consisted of a hollow hemisphere that could fit over the femoral head and create a new smooth surface [6]. A conceptual idea that is similar to the modern femoral resurfacing. Due to the brittle nature of glass the mold arthroplasty never became a success. Inspired by his dentist Smith-Petersen replaced the molded glass with Vitalium®, a cobalt-chromium alloy, and provided the first acceptable results in total hip arthroplasty.

The problem with the first THAs was loosening of the components. An orthopedic surgeon from Whrightington Hospital solved this problem. In 1962, he fixated a metal femoral stem with a small head and a polyethylene acetabular component with acrylic cement. The low friction arthroplasty was born. The surgeon is known as Sir John Charlney, the father of the modern THA [7].

The challenge of today´s THA

Today, THA is an effective treatment for painful advanced osteoarthritis. In 2016, approximately 9,000 (180 per 100,000) primary THAs were performed in Denmark [8,9]. This number is expected to increase [10]. In England and Wales approximately 80,000 primary THAs were performed in 2012. This number is expected to increase up to 800,000 primary THAs pr. year in 2030 [11]. The survival of a primary THA is approximately 80% after 20 years. The main reason for revision surgery is aseptic loosening of the implant [9].

One of today's challenges is the increasing life expectancy. Patients live longer and a relative high number will experience the need for revision surgery [10]. Another challenge is the relative short longevity of THAs inserted in patients less than 60 years of age [9]. Today´s challenges and the future demands emphasize the needs to improve THA longevity.

Optimizing implant longevity

Migration of an implant can be measured with roentgen stereophotogrammetric analysis (RSA), a technique that uses two synchronized x-rays, a calibration cage and small tantalum markers placed in the patient to obtain three-dimensional coordinates of an implant with respect to bone. The technique was introduced by Goran Selvic in 1974 [12]. Several studies using RSA have shown that early implant

migration can predict late aseptic loosening [1–4]. In a meta-analysis with 26 RSA studies, Pijls et al. found that more than 2 mm proximal migration within first 2 years after insertion of an acetabular cup was a strong predictor of late aseptic loosening [1]. Migration less than 0.1 mm was considered safe. A similar association was found in a meta-analysis investigating the association between early migration of shape-closed cemented stems and late aseptic loosing [13].

Literature suggests that an arbitrary threshold for acceptable migration exists for each type of implant exists. This threshold dichotomously divides a population into a group of stable implant with minor or no migration and a group with migration and high risk of later aseptic loosening [14].

One way to improve THA longevity could be through reducing the amount of early implant migration. Accelerating strong and secure fixation of an implant could be one way to reduce the risk of early migration and later aseptic loosening.

Aim of this thesis

The overall aim of the studies included in the thesis was to increase THA longevity and thereby reduce the risk of painful implant loosening and need for revision surgery.

The specific aim of the studies was to improve initial fixation of experimental implants **(I-VI and VIII-X)** or to reduce the effects of an unstable implant **(VII and XI)**. All experiments were conducted with experimental implants placed in either canine or ovine bone. Common for all studies was local treatment with bisphosphonate, a bone anti-resorptive drug. The overall hypothesis was that local treatment with bisphosphonate could increase implant fixation defined by increased biomechanical fixation and osseointegration **(I-VI and VIII-X)** or reduce micromotion induced bone resorption **(VII and XI)**.

Specific hypotheses for each study included in this thesis can be found in the respective article for each study.

Biology and implant fixation

Clinical implant fixation

Total hip arthroplasty (THA) is a successful operation for osteoarthritis. The procedure dramatically relieves pain and improves quality of life [15]. A THA is not a "new hip", but an artificial hip. Implicit in this concept is the recognition that the longevity of a THA is under multifactorial influence by factors such as method of fixation, implant design, surgical technique and quality of host bone [16].

Acetabular or femoral fixation of an implant can be achieved with two different techniques: Cemented or uncemented [16]. The experimental implants included in the thesis are designed to imitate both uncemented **(I-VI and VIII-X)** and cemented **(VII and XI)**.

Cemented fixation

Fixation using bone cement was introduced by Sir John Charlney [7]. Modern bone cement is basically Plexiglas and is made of polymethylmethacrylate (PMMA). In the acetabular socket, cement anchors the acetabular component by binding to the implant surface while interdigitating with trabecular bone [16]. In the femoral canal, cement also acts as filler between the implant and bone. Fixation at the cement-to-bone interface is obtained by having cement interdigitate with the cancellous bone, thus creating a mechanical interlock [17]. At the cement-to-implant interface, two different principles exist: shape-closed and force-closed fixation. Shape-closed fixation requires an implant with a matt surface onto which the cement can bind. Bonding of cement to the implant at the interface holds the implant-cement system together. The implant and cement acts as a composite material where loading forces are transferred from the implant through to bonds at the interface and through the cement mantle into the host bone [17]. Force-closed fixation uses an implant with a polished surface. The implant-cement system is held together by axial loading of the implant taper, thus creating subsidence of the implant and radial compressive forces that are transferred to bone as hoop stress [17]. Due to the viscoelastic properties and stress-relaxation of cement, subsidence of the polished implant can occur without cement fractures. Both shape- and force-closed fixation show excellent implant longevity [18].

Uncemented fixation

While cemented fixation solely rely on mechanical interlock between bone and cement, uncemented fixation rely on initial mechanical fixation between implant and bone followed by biological on or ingrowth of bone to the implant surface [19]. Bony integration of an implant requires stable conditions. These conditions are created by the initial mechanical interlock between the implant and host bone bed [20]. From a clinical point of view, the primary

mechanical stability depends on the implant design (e.g. shape), the surgical technique and patient related factors (e.g. bone quality) [19]. Secondary biological bony fixation depends on the implant surface (e.g. biocompatibility, coating), implant-bone relationship (e.g. no micromovement, no gaps between bone and implant) and patient related factors (e.g. bone healing abilities) [21]. Studies included in this thesis focused on improving or accelerating the secondary biological implant fixation **(I-VI and VIII-X)**.

The surgical bone compaction technique

Several experimental studies have shown the importance of close-fit between implant surface and surrounding bone in order to obtain optimal secondary biological implant fixation [22–24]. Bone is a viscoelastic material [25]. When deformed within the elastic strain boundaries, it has the ability to restore its original shape. One way to optimize the initial bone coverage of an implant surface is by using the spring-back effect of bone [26]. This can be achieved by sequentially expanding the bone bed using smooth instruments and thereby compacting the surrounding bone [27]. This is in contrast to rasping where the cancellous bone is removed. After insertion of the implant, the spring-back effect of the bone will ensure relative high bone coverage of the implant surface [26].

In vivo studies have shown that bone compaction has the ability to increase fixation and osseointegration of cylindrical porous-coated Ti and HA implants [28–30]. However, clinical studies show more conflicting results. One study using bone compaction to enhance fixation of a double wedge femoral stem (Bimetric®, Biomet Zimmer, Warsaw, USA) revealed conventionally broaching superior to bone compaction [31,32]. The inferior results were partly due to peri-prosthetic fractures[31,33]. This is in contrast to the survival rates of the Corail® femoral stem (DePuy Synthes, Warsaw, USA), which uses compaction broaching and has an excellent long-term survival [9]. Furthermore, short-term clinical RSA studies of the Primoris® implant show low early migration [34]. The Primoris® is a short femoral implant inserted into the femoral neck with the use of bone compaction. The conflicting clinical results might be explained by differences in implant design [20].

Bone compaction creates a dense zone of autologous graft around an implant. Experimental studies have shown that some of this graft is non-vital [28,35,36]. Furthermore, the same studies showed that the effect of bone compaction on implant fixation and bone density was diminished over time. A preservation of the non-vital graft might have the potential to prolong and further improve the effect of bone compaction. One way to preserve the non-vital bone could be with the anti-resorptive bisphosphonates. **Study I, II and IV** in this thesis investigated whether local treatment with bisphosphonate was able to preserve this bone and thereby increase implant fixation.

Basic science of implant fixation

Fixation of an implant can be described at different levels: Macroscopically organ level, microscopically tissue level and nanoscale molecular level. Implant fixation at macroscopically level, where the entire bone is considered an organ, depends in part on implant design and mechanical properties of the implant (e.g. stiffness and geometry) [37]. Implant fixation at the nanoscale level describes the specific bonding or direct contact of cells from the bone lineage to the implant surface [38]. The link between the molecular bonding and the fixation at organ level is the tissue level fixation. Implant fixation of **Study I-XI** was evaluated at tissue level. The prerequisite behind long lasting implant fixation is osseointegration [21].

Regeneration of bone around an implant is in many aspect similar to bone healing [39,40]. Preparation of the bone bed and insertion of the implants traumatizes the local tissue and elicits an inflammatory response. This phase has many similarities to formation of a fracture hematoma. After insertion of the implant a blood clot will form. Blood circulation around the implant will be limited the first days after surgery. Signal molecules in the hematoma will attract cells from the immune system. Cells from the bone lineage will be stimulated to begin bone regeneration [39–41]. In the context of reduced blood circulation the first days after surgery, it seems attractive to locally deliver agents intended to stimulate bone regeneration compared to systemic delivery where the reduced vascularity might act as a barrier. This is supported by a rodent fracture study where increased callus formation and mechanical strength was found by postponing the systemic delivery of zoledronic acid compared to delivery at time of surgery [42].

The inflammatory phase is followed by a bone regeneration phase. Cells from the bone lineage are activated. Osteoblasts begin to form woven bone through the process of intramembranous ossification. A prerequisite for intramembranous bone formation is the presence of growth factors, osteogenic cells and a scaffold that guides formation of bone [43].

Growth factors locally are secreted by endothelial cells, platelets, monocytes, macrophages, mesenchymal cells and cells from the bone lineage [44]. One way to facilitate bone regeneration around an implant could be by local delivery of growth factors (e.g. BMP-2). This is part of the rationale behind **Study X** included in this thesis, where BMP-2 was locally delivered. The osteogenic cells are requited from both local sites such as the bone marrow and systemically from the blood circulation in form of multipotent mesenchymal cells [43].

Formation of the new woven bone requires a scaffold. The extracellular matrix in the hematoma and subsequent granulation tissue provides a natural scaffold for bone formation [43]. Another, highly potent scaffold for bone formation is the natural bone autograft created *in situ* during the preparation of the bone bed and insertion

of the implant. During fracture healing bone formation and resorption can occur independently [45]. Both the primary mechanical implant stability and formation of bone in the regeneration phase can be compromised if this autograft scaffold is resorbed to quickly. Prolongation of the autograft resorption might have the potential to facilitate bone formation. The osteoclastic resorption can be inhibited by bisphosphonates [46]. Part of the rationale behind **Study I-III and VIII-IX** was to inhibit or prolong the resorption phase of the local traumatized bone and thereby facilitate bone formation. In situations where the bone bed is insufficient for implantation, primary mechanical stability and scaffold for bone formation can be obtained by the use of bone graft materials (e.g. bone allograft, ceramic bone substitutes) [19]. These bone graft materials face the same resorption problem as the autograft created *in situ*. Part of the rationale behind **Study III, V-VI and X** was to inhibit or prolong resorption of these graft materials.

A prerequisite for bone to bridge from the host bone to the implant surface is stability [19,47,48]. This is similar to the stability needed for bone to bridge two fracture fragments [43,49]. If interfragmentary strain is too high, then bone will not form. In order to achieve secondary biological fixation of an implant, it is important that the primary mechanical implant stability creates a tissue environment with a strain within the range of bone formation. This is supported by several experimental implant studies that have shown that micromotion inhibits bone formation [50–53]. Clinical RSA studies also support that primary implant stability is important for long lasting implant survival [2,4,54]. As described in the previously paragraph, resorption of the supportive bone bed might create an environment with primary mechanical implant instability and too high strain for bone formation to occur. Fibrous tissue can be formed under conditions with higher strain than bone. In settings with an unstable implant, a fibrous membrane will surround the implant instead of bone [52,55]. The rationale behind **Study VII and XI** was to investigate whether local treatment with bisphosphonate could preserve local bone and facilitate new bone formation in an environment with an unstable implant and high interface strain.

The remodeling phase begins after new bone has formed around the implant. In this phase, basic multicellular units (BMU) replace the woven bone with lamellar bone. The BMU is the result of the coupled actions of the bone resorbing osteoclast and bone forming osteoblast. Remodeling of the peri-implant bone will continue lifelong.

Osseointegration

The main goal when inserting an uncemented implant is *osseointegration*. The term osseointegration was first described by Brånemark in 1977 and defined by Albrektsson as direct contact at the light microscope level between living bone and implant [21,56]. Osseointegration is the result of successful bone regeneration and formation around an implant. The definition of osseointegration

is histological and therefore of reduced clinical application. A clinical translation of osseointegration has been suggested:" A process whereby clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading" [57]. Whether or not a THA is rigid fixated can by evaluated with the use of RSA. The ultimate goal of **Study I-VI and VIII-XI** was to improve implant osseointegration. Osteoinduction and osteoconduction are prerequisites for osseointegration.

Osteoinduction

Osteoinduction is the process by which osteogenesis is induced[57]. This means that primitive pluripotent cells are stimulated to develop into the bone-forming lineage. An osteoinductive agent is able to initiate heterotopic bone formation. Osteoinduction can be achieved with the use of growth factors. These growth factors can be produced local in the environment around an implant or added from ex vivo. One of the pioneers behind modern osteoinductive research is Marshall Urist [58]. He was the first to isolate the osteoinductive glycoproteins know as bone morphogenetic proteins (BMPs). BMP-2 used in **Study X** is commercially available and used clinically. Osteoinduction is a requirement for osseointegration.

Osteoconduction

Osteoconduction means that bone grows on a surface [57]. An osteoconductive surface will guide bone growth. An *in vivo* example of an osteoconductive surface is traumatized non-vital bone found around an implant. *Ex vivo* examples are the surfaces of auto- and allogeneic bone graft (**Study III, V and X**). The surface of bone graft substitutes, such as β-TCP granules (**Study VI**) is another example of an osteoconductive surface. Conduction of bone formation does not occur without osteoinduction and a proper blood supply [57]. Presence of an osteoconductive scaffold in the gap between host bone and implant surface will facilitate osseointegration [59].

It is the osteoconductive properties of the peri-implant scaffold that directs bone towards the implant surface. Final osseointegration of a porous coated implant requires ingrowth of bone into the pores of the surface and ongrowth of bone onto the implant surface. Bone conduction or ingrowth into the pores in the implant surface depends on the *biocompatibility* of the material and the pore size [19]. Biocompatibility describes the ability of a material to perform with an appropriate host response in a specific application[60]. Commercially pure (c.p.) titanium is more biocompatible then stainless steel and conducts bone better [57]. Pore size under 100 µm inhibits bone ingrowth [61]. A pore size in the range 100 µm – 400 µm is ideal [19]. Bone ongrowth on a surface depends on the biocompatibility of the surface coating [38].

Implant surface treatments

Osseointegration of an implant depends on the biocompatibility of the implant surface coating. The degree of biocompatibility of an implant surface can by classified

according to the reaction of the host bone [19,62]:
Biotolerant surfaces (e.g. stainless steel, bone cement or Co-Cr) will be surrounded by connective tissue between the host bone and implant surface. No direct bone contact will occur.
Bioinert surfaces (e.g. Titanium and Titanium alloys) can have direct contact with the surrounding bone.
Bioactive surfaces (e.g. Calcium phosphate and glass ceramics) can form direct chemical bonds with surrounding bone. Hydroxyapatite is an example of a calcium phosphate ceramic.

Only implants with bioinert and bioactive surfaces can by truly osseointegrated.

Some of the first clinically used uncemented implants had a smooth surface. However, they had unacceptable failure rates and their use was abandoned [63]. Todays uncemented implant has a roughened or porous coated surface. Grit-blasting or acid etching can be used to roughen an implant surface. Coating a layer of small particles onto the implant can create the porous coating. Different techniques can are used to create the porous coating [62]:
Plasma spraying: heated metal is sprayed onto the implant surface.
Sintering bead technique: small beads are laid onto the surface and bond together to each other and the implant surface with the use of heat.
Diffusion bonding: Titanium fibers are molded onto a titanium alloy surface with the use of low heat and pressure.

Implants used in **Study I-V** had a plasma sprayed porous coating. Implants used in **Study VI and VIII-X** had a porous bead-coated surface obtained by the sintering bead technique.

Implants with a bioinert surface coating (e.g. porous plasma sprayed titanium alloy coating) can be made bioactive by adding a layer of hydroxyapatite (HA). This will increase their biocompatibility and thereby their osteoconductive properties. Plasma spraying an implant surface with HA was first demonstrated in 1987 [64]. Today, HA can by precipitated onto an implant surface. The potential advantage of this technique is a thinner layer HA with relative little effect on the coating porosity. However, both experimental and clinical studies fail to demonstrate a difference between the two techniques with respect to implant fixation [65,66]. Implant in **Study IV, IX and X** were HA coated.

Experimental studies using the same animal model as the studies included in the thesis have demonstrated enhanced mechanical implant fixation and osseointegration of HA compared to porous titanium coatings [48,62,65]. Some clinical studies support the experimental findings while other fail to demonstrate an effect of HA compared to non-HA porous coatings [67–69]. A study from the Nordic Arthroplasty Register Association (NARA) database including 116,069 THAs reveals no clinically relevant effect of HA-coated implants compared to similar non-HA-coated implants [70].

Bone grafts

Primary mechanical implant stability and subsequent biological osseointegration is depended on a sufficient host bone bed. Morselized bone grafts can be used to fill out defects in situations with a compromised and insufficient host bone bed [71,72]. The initial goal of bone grafts is to allow weight bearing and increase primary mechanical implant stability. The subsequent goal is, by time, graft incorporation by host bone followed by total or partial replacement of the graft with host bone. Bone graft incorporation can by histologically defined as revascularization of the surrounding tissue and new bone apposition to the graft fragments [73].

From a clinical perspective, bone grafts can be divided into autograft (autologous bone graft), allograft (allogeneic bone graft), xenograft (xenogeneic bone graft) and synthetic bone graft substitutes [74]. Autograft is considered to be the gold standard [75]. Autograft and allograft can be subdivided according to their histological appearance as cancellous or cortical. Common for autograft, allograft and bone graft substitutes is their *osteoconductive* properties. They all act as a scaffold for bone apposition [74,76]. Resorption of the graft scaffold will reduce the graft surface and might decrease the osteoconductive effect. Preservation of the graft with the anti-resorptive bisphosphonates might facilitate new bone formation. Part of the rational behind **Study III, V, VI and X** was that preservation of the used graft would increase new bone formation. Autograft contains viable cells from the bone forming lineage and is, in contract to all other bone grafts, *osteogenic* [74]. *Osteoinductive* proteins can be found in the extracellular matrix of bone. Autograft and allograft are therefore expected to be osteoinductive. The extent of osteoinductive properties of allograft depends on the method used to process the graft [74]. An example of a xenograft is the osteoinductive demineralized bone matrix.

Graft incorporation depends on several factors. From a biological perspective, graft incorporation describes the interaction between the graft and the host bone leading to bone formation within the graft and adequate mechanical properties [74]. A prerequisite for bone formation is revascularization [74,76]. The dense structure of cortical grafts acts as a barrier for vascular ingrowth. Incorporation of cortical grafts are therefore pronominally mediated by osteoclastic bone resorption follow by osteoblastic bone formation [75,77]. This process is coupled as normal remodeling. The process by which the graft is nearly resorbed and substituted with new viable bone is known as *creeping substitution* [75]. The relative low density of cancellous grafts allows ingrowth of osteoblastic bone formation independently of osteoclastic bone resorption. High graft density slows new bone ingrowth. This is supported by experimental data showing that impaction of cancellous bone grafts impairs new bone ingrowth [78].

Other factors influence graft incorporation. Applying weight transfer to a graft will, all else equal, increase new formation [79,80].

In contrast to autograft, allograft might induce an extended immunologic response. The main part of the cells inducing this response is found in the bone marrow. Rinsing allograft might be one way to improve graft incorporation. However, experimental results are conflicting. One experimental study has shown increased bone formation while another fails to show any benefits [81,82].

The mechanical strength of a morselized cancellous allograft will increase as new bone incorporates the graft. Resorption will preceed new bone formation and creeping substitution will initially occur in areas with high density. The mechanical stability of the graft construct might be compromised if resorption exceeds the bone formation. If the graft resorption could be prolonged, then the mechanical stability might not be compromised. One way to slow down the graft resorption could be with the anti-resorptive bisphosphonates. Part of the rationale behind **Study III, V, VI and X** was to inhibit graft resorption with the use of bisphosphonates.

Failed primary implant fixation

Early migration of an implant, as detected by RSA, is a strong predictor for later aseptic loosening [2,13]. The etiology behind osteolysis and late aseptic loosening is multifactorial [83,84]. It is well-established in the literature that wear particles or particulate debris play an important role [83–85]. Wear particles have in numerous studies been shown to promote bone resorption [83,84,86]. However, wear particles are primary generated at the joint articulation while osteolysis occurs at the implant-bone interface. In order to induce osteolysis at this interface, the particles need to be transported from the joint articulation to the interface. Since, RSA early can predict late loosening and wear particles take time to generate, it seems plausible that other factors initiate the process ending with osteolytic aseptic loosening [83,87]. It has been proposed that mechanical stimuli are of primary importance for aseptic loosening, and that particles play a modulating role in the later stages of implant loosening [83,87].

Implant micromotion could be one primary stimulus of importance for aseptic loosening. Experimental studies have shown that instability can induce bone resorption [51,87,88]. This bone resorption will increase the effective joint space and surround the implant with a soft-tissue membrane [89]. Wear particles generated at the joint articulation can migrate along the soft-tissue membrane to the implant-bone interface and at later stages aggravate the osteolytic response [83]. Another important primary stimulus is fluid pressure. Experimental studies have shown that fluid pressure alone is sufficient to stimulate bone resorption [90–92].

Failed primary implant fixation can occur when the primary stability of an uncemented implant is insufficient to allow secondary biological osseointegration or if the mechanical interlock between cement and surrounding bone is insufficient. The

failed primary implant fixation may not at first be symptomatic for the patient but is will increase implant instability and induce bone resorption leading to later painful aseptic loosening.

The rationale behind **Study VII and XI** was to create of model of failed primary implant fixation and test whether treatment with anti-resorptive bisphosphonates could counteract the instability induced bone resorption. Inhibition of bone resorption would properly not make an unstable implant stable, but might in theory have the potential to reduce formation of a fibrous membrane and thereby counteract transport of particles from the joint articulation to the bone-implant interface. In a clinical setting, this might postpone the final painful osteolytic aseptic loosening.

Bisphosphonates and implant fixation

Basic Science

The first publication to describe the inhibitory effect bisphosphonates on bone resorption dates back to 1969 [93]. Bisphosphonates, initially called diphosphonates, have been known since the middle of the 19th century [46]. They were used in the textile, fertilizer and oil industry to inhibit precipitation of calcium carbonate. Medical studies of bisphosphonates dates back to mid-sixties where Herbert Fleisch, Graham Russell and co-workers persuaded a drug that could inhibit calcium phosphosphate precipitation *in vivo* [94]. They found that inorganic pyrophosphate had that ability [95]. The P-C-P backbone of pyrophosphate allows it to chelate calcium ion and thereby inhibit precipitation. Furthermore, they found that pyrophosphate *in vitro* could inhibit dissolution of calcium phosphosphate crystals. In experimental *in vivo* models, pyrophosphate could prevent ectopic bone formation but had no bone preserving effects. It was destroyed locally by alkaline phosphatases. Bisphosphonates are analogues of pyrophosphate that are not destroyed enzymatically by pyrophosphatases or by hydrolysis in the gastrointestinal tract. Bisphosphonates have the ability to preserve bone *in vivo*. In the first studies, the bone preserving effect of bisphosphonates was ascribed to a physiochemical inhibition of crystal dissolution [46,93]. Later research showed that cellular effects of bisphosphonates, not physiochemical, mediated the inhibitory effect on bone resorption [96].

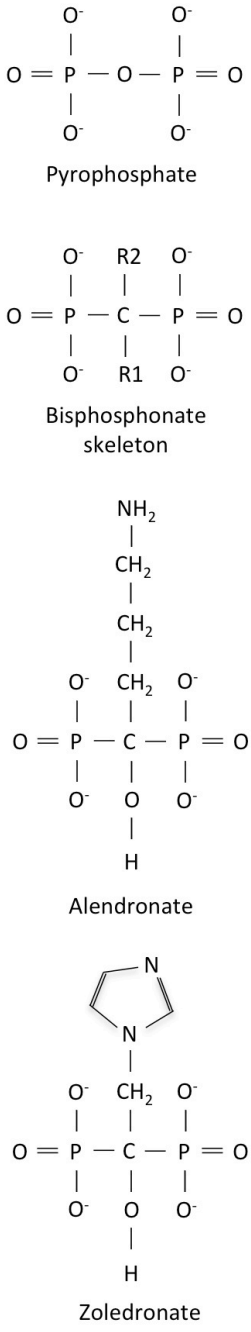


Figure 2. Chemical structures of pyrophosphate, germinal bisphosphate, alendronate and zoledronate

Bisphosphonates are analogues of pyrophosphate. They contain an oxygen atom instead of a carbon atom in the main chain (Fig. 2). Bisphosphonates are divided into two groups depending on which one of the side chains contains nitrogen. The pharmacodynamical mechanisms of the two groups are different [97].

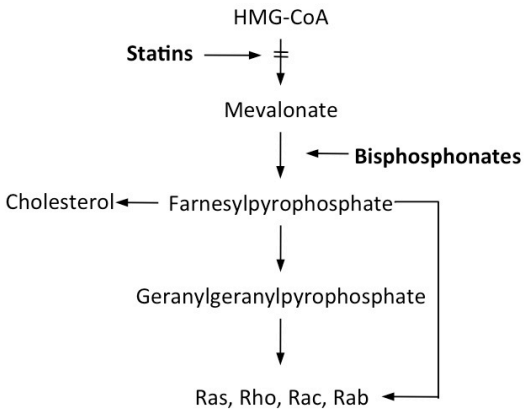


Figure 3. Molecular actions of statins and nitrogen-containing bisphosphonates on the mevalonate pathway. Both drugs inhibit the formation of signal molecules such as Ras, Rho, Rac and Rab.

The non-nitrogen containing bisphosphonates (Non-N-BPs), such as etiodronate and clodronate, closely resembles pyrophosphate and are incorporated into the phosphate chain of ATP. The bisphosphonates containing ATP analogue become non-hydrolysable and thereby inhibits cell function and subsequently leads to cell apoptosis [96]. The more potent nitrogen-containing bisphosphonates (N-BPs), such as alendronate, pamidronate and zoledronate, work by inhibiting the mevalonate pathway [96]. The main target in this pathway is the farnesyl pyrophosphate synthase (Fig. 3). This pathway is used in the intracellular synthesis of cholesterol and other sterols. The end products of the mevalonate pathway are among others used in the posttranslational modification of signal molecular used to control cell function. These signal molecules are fundamental for osteoclast formation and function [94].

ATP synthesis and the mevalonate pathway are found ubiquitously in all cells in the body. However, the effects of bisphosphonates are limited to bone and primarily the osteoclast. This can be explained by the high affinity of bisphosphonates for hydroxyapatite. Once inside the body, bisphosphonates will either adsorb to bone or quickly undergo renal excretion [98]. Bisphosphonates bind preferentially to bone tissue with high turnover where the presence of resorptive surfaces is high [98]. The preference for resorptive surfaces could be explained by the high affinity for hydroxyapatite that is exposed at these sites. The chemical bond between BPs and the mineral phase of bone is under physiological pH almost irreversible. However, during bone resorption the osteoclast lowers the pH in the resorption lacuna. The acidic pH causes the bisphosphonates to dissociate from the mineral surface. Free bisphosphonates in the subosteoclastic space are internalized into the osteoclast by endocytosis [99].

Some *in vitro* studies have revealed that bisphosphonates can stimulate osteoblast formation [100,101]. However, other studies indicate that concentrations of bisphosphonate unlikely to occur *in vivo* are needed [102,103].

Today, bisphosphonates are, due to their

anti resorptive properties, used in established clinical treatment protocols against osteoporosis, Paget´s disease, hypercalcemia of malignancy and malignancies with metastasis to the bone [104,105]. Although preclinical and clinical results are promising, the role of bisphosphonates as an established adjuvant in clinical implant fixation still awaits.

Bisphosphonates and experimental implant fixation

Osseointegration of uncemented implants is dependent on a stable mechanical primary fixation at the implant interface. A too high osteoclastic bone resorption around the implant might compromise the primary stability. Bisphosphonates can inhibit osteoclastic bone resorption and have the potential to increase the primary mechanical and secondary implant fixation. This is the overall rational for using bisphosphonates as an adjuvant for implant fixation in **Study I-VI and VIII-X** and in many of the studies described in the literature.

Systemic delivery of bisphosphonate

In the context of implant fixation, bisphosphonates exerts their effects at the implant-bone interface and peri-implant bone. One way to deliver bisphosphonates to these sites is by systemic administration. Several studies have investigated the effect of systemic delivery of bisphosphonates on implant fixation and osseointegration [106–112]. A general finding was increased implant osseointegration. This effect was explained by a preservation of the supporting bone bed and newly formed bone. None of the studies found impaired implant fixation compared to the control implant. However, some of the studies had subgroups were treatment with bisphosphonate did not statistically influence implant fixation [106,108,112].

Timing of bisphosphonate administration is an important factor. A too early administration will be of reduced effect since the implant is surrounded by a hematoma. A too late administration will also be of reduced effect since osteoclastic resorption already has begun. This is supported by a study investigating the effect of a single dose of zoledronate administrated systemically at three different time points on fracture healing. Delaying administration 1 and 2 weeks after creating the fracture increased callus size and strength [42].

Local delivery of bisphosphonates

One way to overcome the challenges of systemic delivery is by local administration of bisphosphonate. The bisphosphonate can be applied locally either from a coating on the implant surface or by locally soaking the bone bed. Advantages of local administration are the ability to obtain optimal concentrations of bisphosphonate at time of surgery. Animal studies have shown that bisphosphonates remain highly localized after topical administration [113,114].

A simple way to locally apply bisphosphonate is by soaking the bone bed in a bisphosphonate solution. Beside **Study I-II, IV and XI** in this thesis only few experimental studies have investigated this method of administration [115,116]. Both studies found that topical bisphosphonate reduced resorption of the bone bed. Furthermore, one of the studies investigated implant osseointegration and found that soaking the bone bed with bisphosphonate increased mechanical fixation of the implant [115].

Another way to locally apply bisphosphonates to the peri-implant bone is using a surface coating. Several experimental studies have investigated whether local administration of bisphosphonates from the implant surface will stimulate osseointegration [117–127]. General findings are improved implant osseointegration and fixation. None of the studies find negative effects on implant fixation. Two of the studies used a poly (D, L-Lactide) coating to deliver zoledronate locally. The same coating was used in **Study VIII and IX**. Some of the studies investigated different surface concentrations of bisphosphonate and found a dose-dependent effect [119,120,127]. Peter et al. found their intermediate dose to be most effective in terms of implant fixation [127]. This indicates that an optimal dose exists for each bisphosphonate and that a too high dose can have negative influence on implant fixation.

Bone allografts and bisphosphonates

Bone allografts are used in situations with reduced bone stock. Allograft has many similarities to the traumatized and necrotic bone surrounding an implant. Osteoblastic bone formation is metabolically demanding and requires adequate blood supply, while osteoclastic bone resorption is less dependent on vascularization [128]. Due to the reduced vascularity of newly implanted allografts, there might be an imbalance between graft resorption and bone formation. Bisphosphonates might protect allograft against this resorption. Studies using a rodent experimental bone chamber filled with allograft have shown that both systemic and local treatment with bisphosphonates can protect the graft against resorption[129–135]. The strongest response was found when bisphosphonate was applied locally instead of systemically [133]. The same results with respect to preservation of the graft are found in studies using a canine model similar to the design of **Study III, V, VI and X** [136–138].

Treatment with bisphosphonates not only protects allograft against resorption, but it also increases new bone formation within the graft. Several studies have found increased new bone formation with allograft treated with bisphosphonates [129,131,132]. The effect might be due to reduced osteoclastic activity and retention of newly formed bone [131].

New bone formation within an allograft is dependent on the density of the graft. Formation of bone within impacted grafts

with a relative high density is dependent on osteoclasts to make space for new bone. Jeppsson et al have shown that impacting bisphosphonate soaked allograft impairs new bone formation [135].

Bone morphogenetic proteins (BMPs) stimulate bone formation and allograft remodeling [139–141]. The increased graft resorption observed with BMPs might be counteracted with the use of bisphosphonates. Conflicting results are found in the literature. Some studies finds that the combination of BMPs and bisphosphonates preserves graft and increases new bone formation [134,140]. Other studies find that the combination of BMPs and bisphosphonates inhibit new bone formation [136,138].

Bisphosphonates and osteolysis

The etiology behind osteolysis is complex [84]. The osteolytic cascade ends in peri-implant bone resorption and often a loose implant. Bisphosphonates might prevent or slow down the osteolytic process by inhibiting the bone resorption. Astrand et al. have shown that systemic and local bisphosphonate treatment can reduce but not prevent instability and pressure-related bone loss [53,116,142]. Other studies have investigated whether bisphosphonate influences particle-induced bone resorption. Von Knoch found in a murine calvaria osteolysis model that systemic bisphosphonate could reduce particle-induced osteolysis [143]. The same results are found in both a rodent and a canine model of particle-induced osteolysis [144,145].

Bisphosphonates and clinical implant fixation

Only few studies have investigated the effect of bisphosphonates on clinical implant fixation. Hilding et al. have shown that systemic treatment with clodronate reduced migration measured with RSA of a cemented total knee prosthesis [146,147]. The same results were found when applying ibandronate locally [148]. Soininvaara et al found the systemic bisphosphonate treatment with alendronate was able to reduce the postoperative bone loss measured with DEXA around total knee implants [149]. Wilkinson et al. found that systemic treatment with pamidronate did not influence migration of acetabular cups measured with EBRA software, but did reduce bone loss measured with DEXA around the femoral stems [150]. In contrast to Wilkinson et al, Schilder et al found that soaking the acetabular socket in ibandronate reduces acetabular cup migration [151]. They used a cemented cup and measured migration with RSA.

Two studies have investigated the effect of soaking morselized allograft in bisphosphonate in the context of impacting grafting and revision surgery of THAs. Kesteris and Aspenberg found that soaking allograft in ibandronate and impacting it into the femoral canal preserved the bone mineral density measured with DEXA [152]. Zampelis et al. found that allograft soaked in clodronate and impacted into the acetabular socket both preserved bone mineral density as measured with DEXA

and reduced cup migration measured with RSA [153].

Epidemiological studies using national joint registries indicate that long-term use of bisphosphonates reduces the risk revision surgery for aseptic loosening [154,155]. However, none of the studies are able to clarify whether the association is causal.

Consideration on methods used to study implant fixation

Experimental models

Study design

All studies included in this thesis were designed as paired studies with intervention and control implants in the same animal. An overview of the study design of each study included in this thesis can be found in "Thesis at a glance". The implants were either placed in the proximal metaphysis of the tibia or the humerus or the distal epiphysis of the femur. The paired design reduces inter-animal variation and allows a lower number of animals needed without reducing the statistical power. The drawback of having the control and intervention implant in the same animal is systemic distributing of bisphosphonate from intervention implant to control implant. Studies have shown that locally applied bisphosphonate remains highly localized [113,114].

In **Study I and II**, symmetry between left and right side of the test animals was assumed. The control implants were inserted in left side of the animals and intervention implants in the right side of the animals. In was assumed that fixed placement of the implant would reduce the risk of confusing the implants with each other during surgery and subsequent specimen preparation. Geometrical symmetry of the canine femur has previously been described [156]. During each gait cycle, the test animal transfers load thought the implants. Little is known whether dogs and sheep symmetrically load their bones. In order to eliminate any effect of asymmetrical loading and difference in local tissue reactions to implants, it was decided in **Study III-XI** to alternative between left and right side when inserting control and intervention implants. Placement of the implants in the first test animal in each study was determined by randomization. In studies with four treatment groups, the implantation site was alternated systematically with random start between the different groups.

In all studies, the control implants were inserted before the intervention implant with bisphosphonate. This was done in order not to contaminate the control implants with bisphosphonate. This implies that surgery was not done blinded. Subsequent specimen preparation, mechanical testing and histomorphometry were done blinded.

The small size of the implants and the large size of the animals allow multiple studies to be conducted in the same series of animals. The dog allows implant studies to be conducted simultaneously in the metaphysis/epiphysis of the humerus, femur and tibia. The sheep allows studies in the humerus and femur to be conducted simultaneously. From an ethical and economical point of view this seams correct. A drawback of several studies in

the same animal is systemic effect modification of one study on the remaining studies. All studies included in this thesis were conducted in animals included in multiple studies. These other studies did either investigate the effect on different surgical techniques, different surface coatings or locally applied adjuvant therapies for bone grafts on implant fixation [157–164].

Experimental animals

The choice of test animals is highly dependent on the research question asked. If the purpose of a given study is to investigate gene expression at an implant-bone interface, then a rodent model would be useful given the availability of genetic probes for this group of animals [165]. If the purpose of a given study is to investigate the interaction between implant and bone at tissue level, defined by implant osseointegration and mechanical fixation, then a large animal would be appropriate. The canine bone closely resembles human bone with respect to structure and composition [166]. Furthermore, the size of the canine bones allows paired insertion of multiple implants in metaphyseal bone, thereby elimination statistical inter-animal variation. The dog was used as test animal in **Study I-VI and VIII-X**. The dog is an expensive test animal. The sheep is less expensive, but still with a bone structure that resembles humans [166]. The sheep is easy to handle and was used as test animal in **Study VII and XI**.

All animals used in this thesis were skeletally mature. All experiments were approved by the Local Institutional Animal Care and Use Committee (IACUC) at the Minneapolis Medical Research Foundation in Minneapolis, USA (**Study I-VI and VIII-X**) or by the Danish Animal Research Inspectorate (**Study VII and XI)**. The animal studies were conducted according to the ARRIVE guidelines.

Sample size

The primary outcome used for sample size calculation was biomechanical implant fixation. Sample size for a paired study was calculated using the following formula:

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

$$7.8 = \frac{(1.96 + 0.84)^2 x\ 50^2}{50^2}$$

n = number of animals
$z_{1-\alpha/2}$ = the (1-α/2) percentile in the z-distribution at two-sided testing
$z_{1-\beta}$ = the (1-β) percentile in the standard distribution
SD_{diff}^2 = square of the standard deviation on the paired differences
d^2 = square of the minimal relevant difference

Risk of first (α) and second (β) kind was assumed to be 5 % and 20 % respectively. Based on previous studies using the same implant model, standard deviation on the paired difference (SD_{diff}) for relative improve in mechanical fixation was assumed to be 50%[28,29,62]. The minimal relevant difference (d) was set to an

improvement of 50% in mechanical fixation.

Based on the sample size calculation, 8 animals were included in **Study I**. Two extra animals were included in **Study II-XI** to counteract for loss of animals.

Implant models

The implant models used in this thesis are well established [62]. The models are designed to imitate either an uncemented or cemented implant placed in cancellous bone. The models allow the implants to be surrounded by a gap and/or direct weight bearing if needed. The gap can be filled with bone graft or graft substitutes. Furthermore, the direct weight bearing implants can be made controlled unstable in order to imitate a loose joint prosthesis.

All models are designed to study early fixation of implants. Common to all models is the simple cylindrical implant with a height of 10 mm and a diameter between 5.6 mm and 8 mm. The experimental implant surface replicates the surface of clinical used prostheses. All models are relative simple and highly reproducible. The paired design eliminates inter-animal

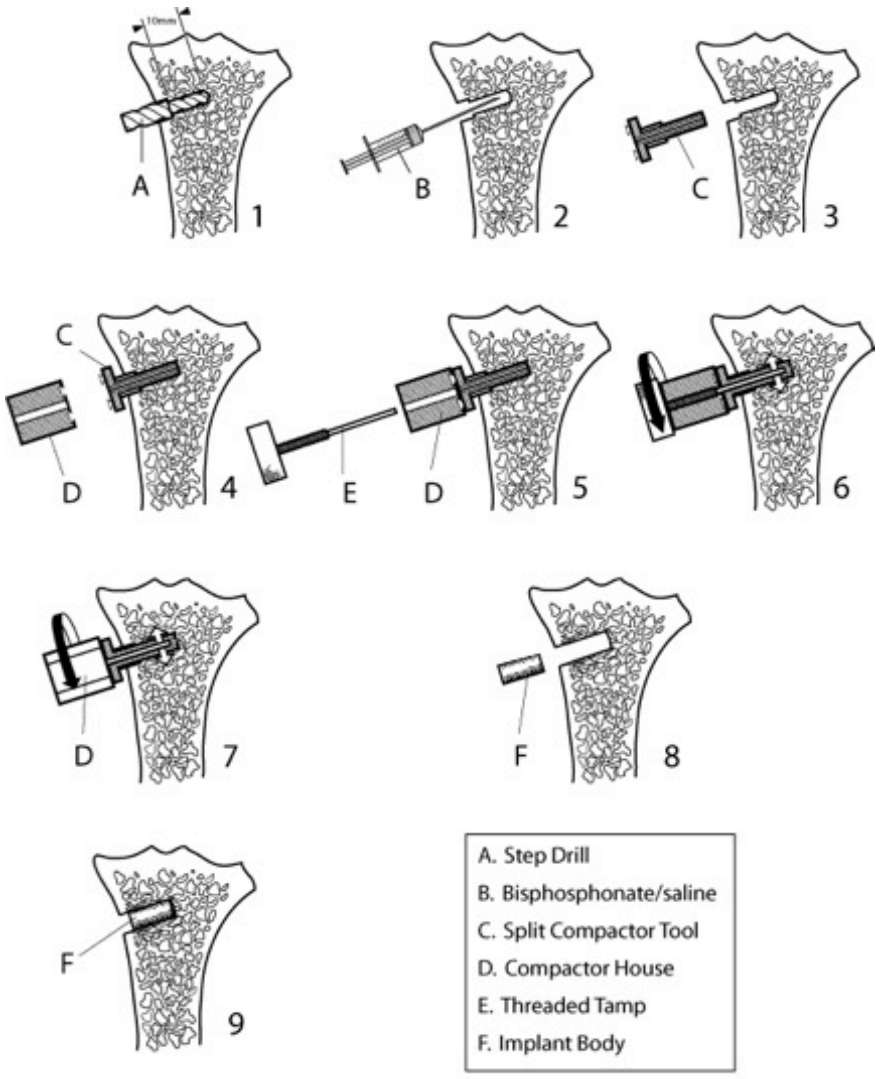


Figure 4. Schematic diagram showing the steps in the bone compaction technique in tibia.

variation. The use of dog or sheep as test animal increases the external validity when translating results into a clinical setting compared with rodent implant models. All test animals were young and healthy and thereby with a high potential for successful implant osseointegration without any adjuvant treatment. A potential benefit of a treatment could therefore be masked by the already optimized healing potential of the animals. The trade-off for the simplicity of the non-weight bearing models is the absence of clinical relevant influence such as direct weight bearing and joint fluid.

The bone compaction model (Study I-II and IV)

The purpose of the bone compaction technique is to secure that the implants are placed in extreme press-fit in cancellous bone. The compaction technique gradually expands the bone cavity by compacting the surrounding bone bed. While compaction the surrounding bone a zone of autologous autograft is created. Compacted bone is known to have a spring-back effect [26]. This implies that initial mechanical fixation of inserted implants will be increased due to the viscoelastic properties of the bone bed.

The compacted bone bed is made by gradually expanding the bone cavity with custom-designed instruments (Fig. 4 + 5).

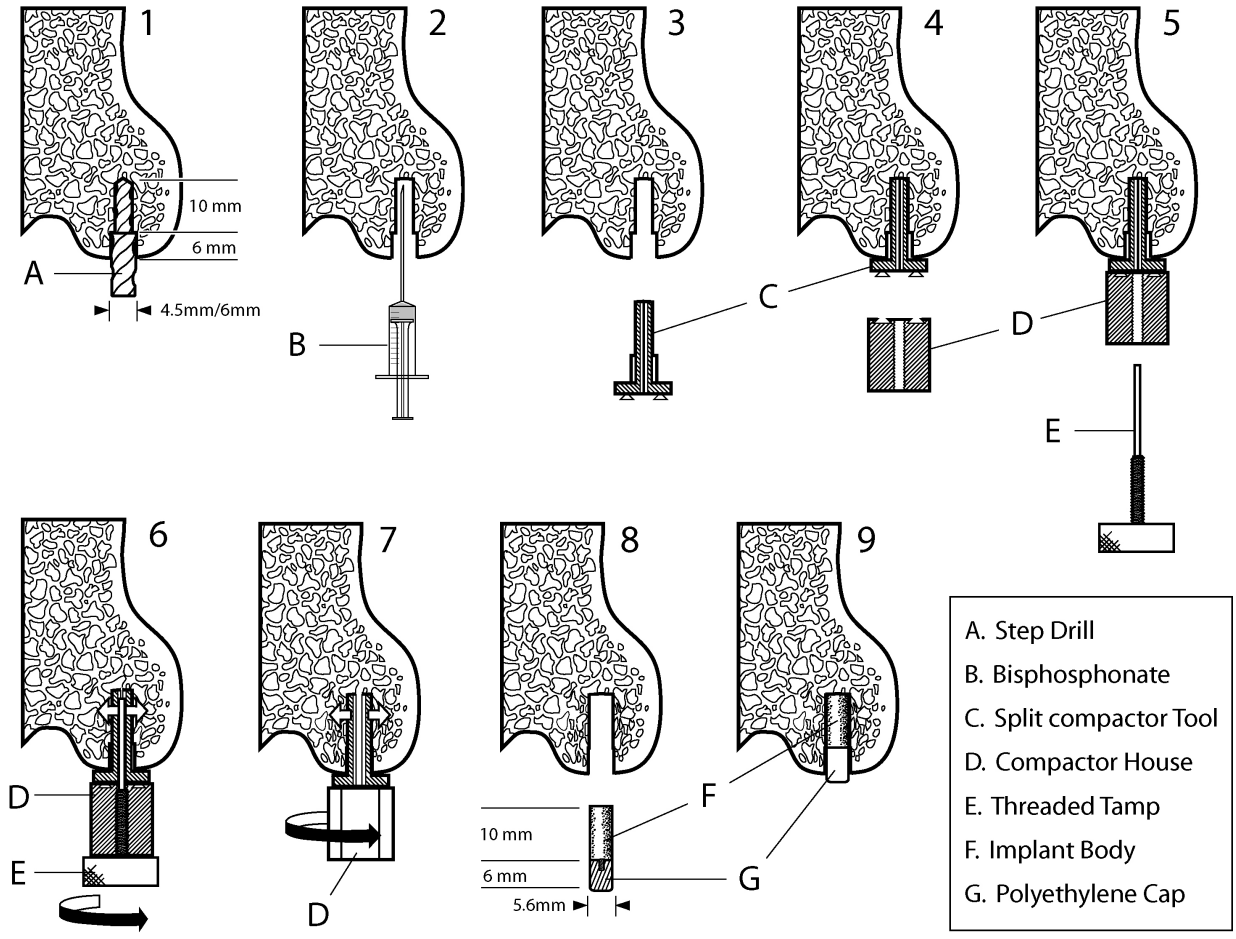


Figure 5. Schematic diagram showing the bone compaction technique in femur.

Figure 7. Gap implant.

The purpose of **Study I-II and IV** was to investigate the effect of alendronate on implants inserted with bone compaction. On the intervention side, the bone bed was soaked in 5 mL alendronate solution (2 mg alendronate per 1 mL saline)(Merck Sharp and Dohme, West Point, PA, USA) for 2 minutes before compacting it. Saline was used as control on the contralateral side. Implants inserted with bone compaction were tested under two different loading conditions. **Study I** investigated the effect of alendronate on direct weight bearing implants inserted bilaterally into the medial femoral condyles (Fig. 5). These implants were inserted intra-articular with direct access to joint fluid. **Study II and IV** investigated the effect of alendronate on non-weight bearing implants inserted bilaterally into the proximal part of tibia (Fig. 4). Implants in **Study I and II** had a plasma sprayed porous-coated titanium alloy surface while implants in **Study IV** had an additional layer of hydroxyapatite.

Grafted gap model (Study III, V-VI and X)
The grafted gap model allows investigation of the effects of different bone grafts or graft substitutes in conjunction with adjuvant therapies on implant fixation and

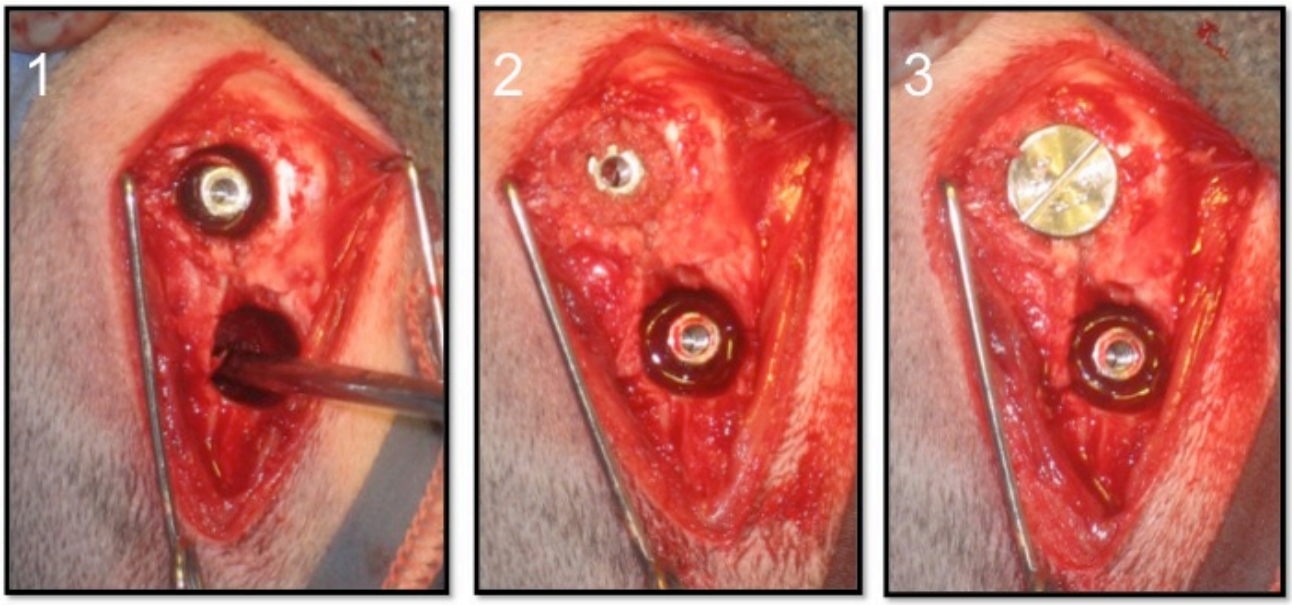


Figure 6. Impaction of allograft around gap implant. 1. Implant. 2. Allograft impacted. 3. Gap closed with end cap.

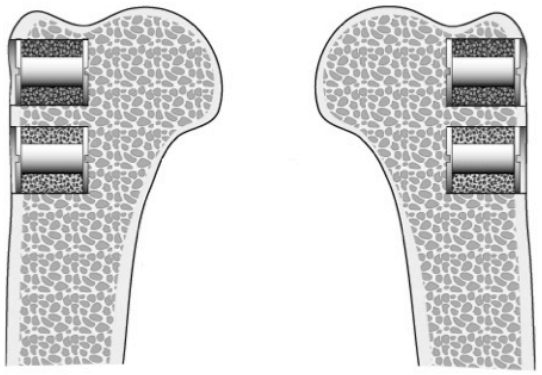


Figure 8. Gap implant in the proximal part of humerus.

osseointegration. The implants used in the grafted gap model are surrounded by a 2.5 mm concentric gap created by two end caps. All implants and end caps had a diameter of 6 mm and 11 mm respectively (Fig. 6). Different surface treatments can be applied to the implants. The gap is filled with either morselized impacted allograft (**Study III, V and X**) or a bone graft substitute (Fig. 7). Tricalcium phosphate granules were used in **Study VI**. Placement of the implants in the proximal part of the humerus created a four-armed study with two implants on each side (**Study III and V**) (Fig. 8). The same four-armed study design can be obtained when placing the implants in the distal part of the femur at

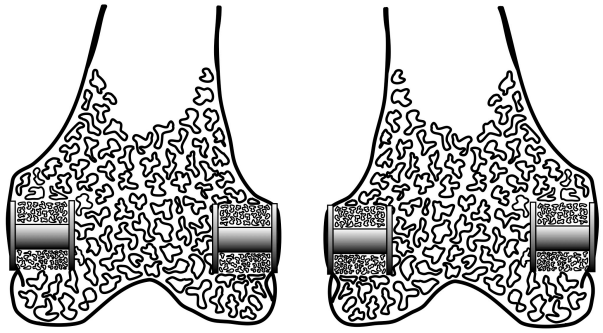


Figure 9. Gap implant in the distal part of femur.

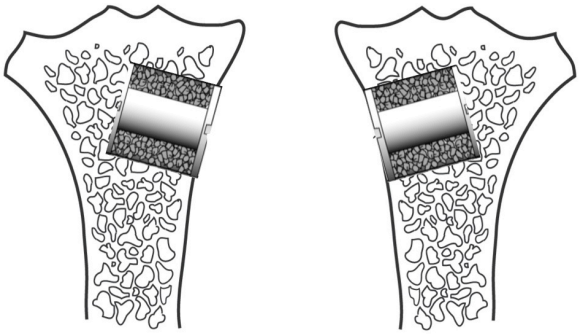


Figure 10. Gap implant in the proximal part of tibia.

the level of the epicondyles (**Study X**)(Fig. 9). The strength of the four-armed design is inclusion of four treatment groups in the same animal. The drawback is the risk of direct contamination between implants in the same bone. In **Study VI** the implants were placed in the proximal part of tibia (Fig. 10). Due to the size of the tibial metaphysis only one implant can be inserted into each bone. Hence only two treatment groups were included in **Study VI**.

During surgery the implants were inserted into an 11-diameter hole with a depth of 12 mm. Concentrically placement of the implant is secured by an end cap with a diameter of 11 mm mounted on the profound end of the implant. The graft was impacted around the implant and secured in the gap by an end cap mounted on the superficial end of the implant (Fig. 7). The graft material was soaked in a bisphosphonate solution or saline (control) before implanting it.

Exact-fit implant model (Study VIII and IX)
The exact-fit implant model is one of the simplest models used in the thesis. It is designed to test the effect of a given

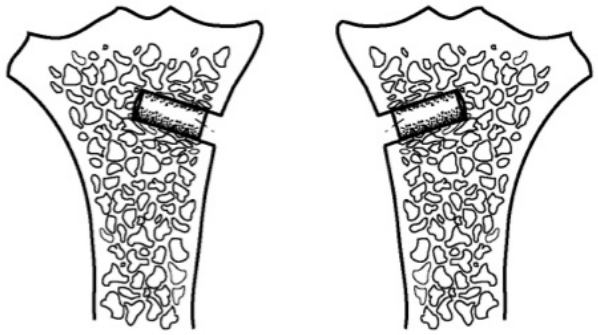


Figure 11. Exact-fit implants in the proximal part of tibia.

implant surface on implant fixation. The model allows immediate and direct interaction between the implant surface and surrounding bone. Cylindrical implants with a diameter of 6 mm was inserted bilaterally into the proximal part of the tibia (Fig. 11). The implants were inserted into a bone cavity with a diameter of 6 mm. On one side an intervention implant was inserted. On the contralateral side the control implant was inserted. **Study VIII** tested the effect of a surface coated titanium coated implant with PDLLA containing zoledronate. Hydroxyapatite coated implants were used in **Study IX** instead of titanium coated implants.

Model of failed initial implant fixation (Study VII ad XI)

The Soballe group has previously developed an implant model that creates conditions imitating a revision setting with an unstable implant, a fibrous membrane and polyethylene wear debris [55]. The implant model used in **Study VII** was a modification of the revision implant model. The idea behind the model used in **Study VII** was to create an implant model that imitated an implant with inferior initial fixation. Loading of the implant should result in micromotion at the interface and thereby create conditions similar to an implant with a failed initial implant fixation. The implants in the **Study VII and XI** were, in contrast to the revision model, placed in exact fit. This was done in order to imitate a primary cemented implant with close contact between the cement and surrounding bone. The implant was made of PMMA and had a smooth surface. This allowed the implant to be placed in exact fit with the surrounding bone and still be able to move when loaded.

Controlled micromotion of the implant was obtained by using the custom-made micromotion device from the revision implant model. The micromotion device consists of an anchor that contains a spring-loaded piston (Fig. 12). The PMMA

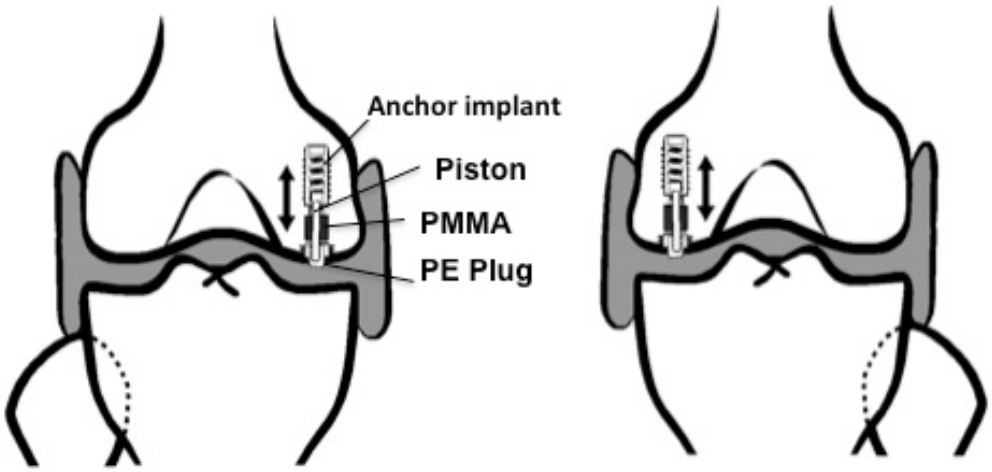


Figure 12. Micromotion implants in the medial femoral condyle.

implant and a polyethylene plug are screwed onto the piston. During each gait-cycle the micromotion device allows the PMMA implant to piston 0.5 mm.

Implant characteristics

Table 1 gives an overview of the different implants used in this thesis. All uncemented implants used in this thesis consisted of a custom-made titanium alloy (Ti-6Al-4V) core. Two different porous coatings were used. A porous titanium alloy (Ti-6Al-4V) coating deposited with the plasma-spray technique by Biomet Inc. (Warsaw, IN, USA), or a porous coating consisting of pure titanium beads sintered together by DePuy Inc. (PoroCoat®, Warsaw, IN, USA). The porous plasma-spray coating had a mean pore size of 480 µm and a porosity of 44 %. The porous bead coating had a mean pore size of 275 µm and a porosity of 45 %. Implants used in **Study IV, IX and X** had an additional layer of hydroxyapatite applied on top of the porous coating. The hydroxyapatite layer had an average thickness of 50 µm and was applied with the the plasma spray technique. All surface coatings used in this thesis were applied using the same techniques used on commercial available implants. Data on implant surface characteristics were supplied from the manufacturer.

Implants used in **Study VII and XI** were custom-made from molded PMMA and did not have any additional surface coatings.

PDLLA surface coating

Study VIII and IX used a poly (D, L-Lactide) (PDLLA) coating as drug carrier. PDLLA is a lactic-based polymer. Lactic acid is released from the coating by hydrolysis and metabolized by Krebs cycle

Tabel 1: Overview of implants used in this thesis.

Study	Material	Height (mm)	Diameter (mm)	Coating	Additional coating
I	Ti	10	5.6	PS	-
II	Ti	10	8.0	PS	-
III	Ti	10	6.0	PS	-
IV	Ti	10	8.0	PS	HA
V	Ti	10	6.0	PS	-
VI	Ti	10	6.0	PC	-
VII	PMMA	10	7.5	PMMA	-
VIII	Ti	10	6.0	PC	-
IX	Ti	10	6.0	PC	HA
X	Ti	10	6.0	PC	HA
XI	PMMA	10	7.5	PMMA	-

Ti = Titanium

PMMA = polymethylmetacrylate

PS= Plasma sprayed

PC= PoreCoat

HA = Hydroxyapatite

into water and carbon dioxide. Implants in **Study VIII and IX** were coated using the protocol described by Greiner et al [124,167,168].

Pure zoledronate (Novartis Pharma AG, Basel, Switzerland) was dissolved in poly (D, L-Lactide) (PDLLA) – Resomer 203 (Boehringer Ingelheim GmbH, Germany) and ethyl acetate solution in a 2% w/w ratio of zoledronate to PDLLA. The coating was applied to the implants by dipping them in the solution twice. All handling of implants and subsequent air-drying was done under sterile conditions.

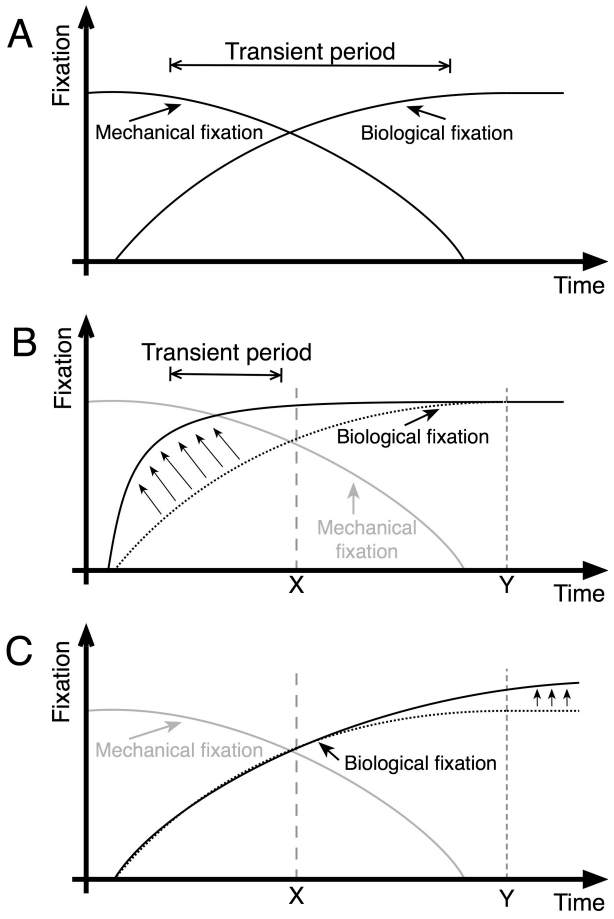


Figure 13. A: Transition from mechanical fixation to biological fixation. B: Accelerating biological fixation. C. Improving longterm biological fixation. X = Ideal observation time to study early events. Y = Ideal observation time to study longterm effects.

Based on difference in implant weight before and after coating procedure, an estimated average dose of 0.02 mg zoledronate was incorporated into the PDLLA coating. It has previously been shown in vitro that 90 % of the zoledronate will be released within the first 24 hours [117].

Bone graft materials

Morselized allograft was used in **Study III, V and X**. Allograft for each series of surgery was prepared in a single session. Graft was harvested from two dogs not included in the studies. The proximal part of the humerus, distal part of the femur and proximal part of the tibia was used. Soft tissue and cartilage were removed from the bones and fine grains with a size of 1.0 – 2.5 mm were made using a bone mill. All allograft grains were mixed in order to even out potential differences in bone quality. The allograft was packed into small portions and stored at -80°C.

The commercially available bone graft substitute, Conduit, was used in **Study VI**. Conduit (DePuy Inc., Warsaw, IN, USA) is made of pure β-TCP. It comes in form of granules with a size of 1.5 mm – 3.0 mm. The porosity is approximately 70 %.

Observation time

Initial fixation of an uncemented implant is mechanical. By time the supporting bone bed will due to the viscoelastic properties undergo stress relaxation and the implant becomes mechanical loose. In the living

bone, the fixation of an implant is maintained due to biological osseointegration of the implant (Fig. 13A). A transient decrease in implant fixation can be observed in the transition from mechanical to biological fixation[169]. This decrease can potential be delirious for the long-term implant survival, if the reduced fixation allows micromotion at the interface.

Implant fixation is a temporal process and a single observation period only describes a cross section of these events. Choosing the optimal observation period is important.

The optimal observation time of a study depends on the purpose and question asked. In situations were the purpose is to reduce the transient decrease in fixation between mechanical and biological fixation, then the observation time must correspond with the transition period (Fig. 13). If the observation time is too short or long, then no effect of treatment will be found.

If the purpose of an intervention is to acceleration biological osseointegration, then the observation time must correspond to the early transition period (Fig. 13). A too long observation period might not show any effect of treatment.

Evaluation of treatments intended to increase the final implant fixation needs an observation period longer that the transition period. A too short observation period might not show any difference although the final implant fixation is increased.

The observation periods for implants included in this thesis were observation for either 4 or 12 weeks. Studies using the same implant model and observation periods have been able to show effect of a treatment [23,29,36,170]. However, all studies included in this thesis are limited since only one or two observation periods are included. With only one observation period it is not possible to conclude which stage in implant fixation is evaluated.

Specimen preparation

A bone block containing implant and surrounding bone was immediate post mortem stored at -20°C. After thawing the bone block, two specimens were cut perpendicular to the long axis of the implant (Fig. 14). Only one specimen was cut from the implants in **Study VII and XI**, since no mechanical analysis was performed. The most superficial specimen

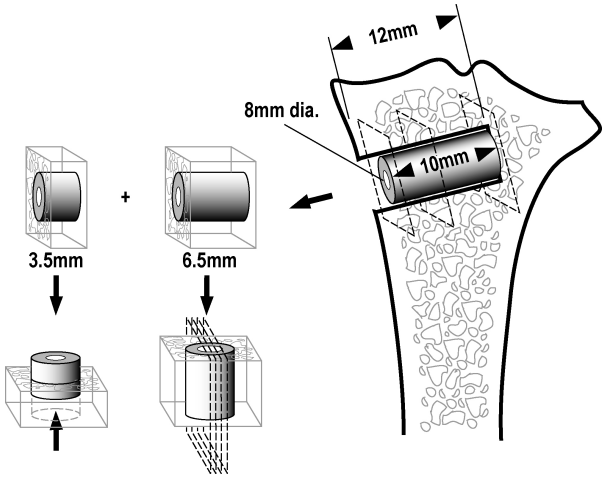


Figure 14. Specimen preparation. Each bone-implant specimen is cut into two pieces. 3.5 mm for mechanical testing and 6.5 mm for histomorphometrical analysis.

was stored at -20°C for later mechanical testing. The remaining specimen was fixed in 70 % ethanol and embedded for later histomorphometrical analysis. Bone specimens from **Study VII and XI** were only prepared in 70 % ethanol for histomorphometrical analysis.

The used preparation method implies that specimens for mechanical testing are frozen and thawed twice. It has been shown that freezing can affect the viscoelastic properties of trabecular bone, but not cortical bone [171,172]. The preparation method can therefore potentially influence the results from the mechanical testing. The paired study design does however imply that specimens are equally affected.

Mechanical and histomorphometrical analysis were done on two different parts from each implant. It is an advantage of the model that both analyses can be performed from the same implant. Using histomorphometrical data to explain mechanical data could be biased since the two specimens are spatially separated. However, it is important to note that paired statistical comparison of both mechanical and histomorphometrical data always were done between corresponding parts of the implants.

Mechanical testing

The overall purpose of the studies in this thesis was to increase the secondary biological implant fixation. This fixation can be evaluated using different tests. In this present series of studies, mechanical fixation was evaluated using a destructive push-out test. Examples of other destructive tests are the pull-out and removal torque tests. Bone is an anisotropic material[173]. This implies that different values of implant fixation can be expected when testing fixation in different directions. A pull-out test would e.g. be suitable for testing adhesion strength between two materials. A removal torque test would e.g. be suitable for testing shear fixation of a screw or angular stability of a femoral stem in cadaver bone. The rational behind choosing a push-out test in this thesis was to imitate the primary directing of interface loading of a prostheses *in vivo*. The applied test primary evaluates shear forces between implant and bone at the interface. However, due to interdigitating between bone and the porosity of the implant surface, compressive and tensile forces will also be included in the test.

An alternative to the used destructive push-out test is a non-destructive test [174]. A non-destructive test preserves the bone-implant interface for later histological evaluation. The limitation of the test is that it only evaluates shear modulus and not includes evaluation of the fixation strength [174]. A non-destructive test does however more closely imitate the clinical loading of an implant due to the cyclic loading during testing.

Implants in **Study I and II** were evaluated on an Instron Universal Test Machine (Instron Ltd., High Wycombe, UK). Implants in **Study III- VI and VIII-X** were evaluated on a MTS Bionics Test Machine (MTS, Eden Prairie, MN, USA). A 10 kN load cell was used for testing. Implants from

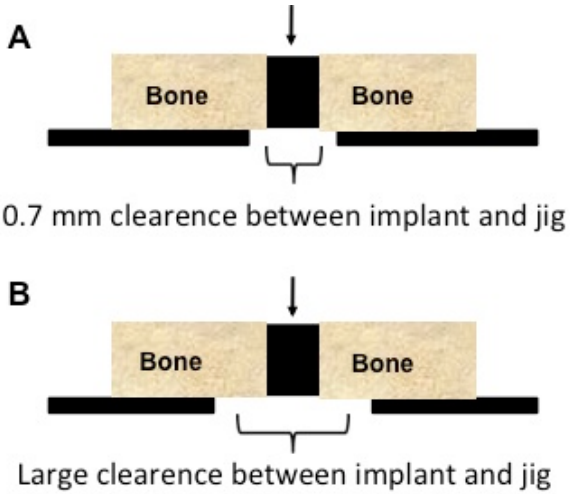


Figure 15. Bone specimen on supporting jig before push-out test.. A: 0.7 mm clearence between implant and jig. B: Large clearence.

Study VII and XI were not evaluated mechanically.

Bone-implant specimens were during testing placed on a metal support jig. The diameter in the jig opening was 1.4 mm larger than the implant diameter. Specimens were thawed for one hour prior to testing. Testing was done blinded and in one session for each study. The direction of implant displacement was from the superficial side and towards inside of the bone. A preload of 2-3 N defined the start of the test. A displacement rate of 5 mm/min was used. A computer recorded a continuous force-displacement curve. Reproducibility of the test was not possible due to the destructive nature of the test.

Influence of test conditions on results

Bone is a viscoelastic material [175]. The strength is therefore dependent on the deformation rate [175]. A relative low deformation rate of 5 mm/min was used in order to reduce the impact of the deformation rate on obtained results. Furthermore, the viscoelastic properties of bone are dependent on its content of water. It is therefore important that testing is done of non-frozen specimens.

It has previously been shown that the clearance of the hole in the supporting jig is important for occurrences of peak stresses [176,177]. A clearance of at least 0.7 mm has been recommended [177]. The purpose of the mechanical push-out test is to evaluate the fixation at the implant-bone interface. Increasing the clearance will allow distribution of load from the implant into in a larger volume of the peri-implant bone before being transferred to the supporting jig (Fig. 15). Increasing the clearance will therefore include testing of the bone further away from the implant surface. A clearance of 0.7mm was used in this thesis, since the main focus was to test the mechanical properties at the interface. However, it could have be of interest in the

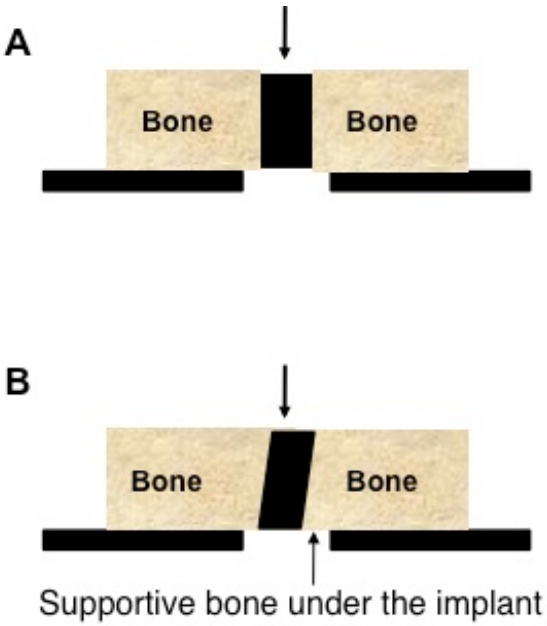


Figure 16. A: Correctly prepared implant. B: Increased force is needed to displace the implant due to the supportive bone.

allograft studies to include the gap inside the clearance. By doing this, the "chain" connecting the implant with the bone bed would have been tested.

During testing it was assumed that displacement of the implants was parallel to the surface (Fig. 16). If a bone-implant specimen during preparation was not cut perpendicular to the long axis of the implant, then overestimation of fixation can be introduced. The implant will require increased load to displace due to the supportive bone. Preparation was done blinded, and it was assumed that implants prepared suboptimal were evenly distributed between the treatment groups. This will not constitute a bias, but instead increase the variance of data.

Mechanical parameters

Three mechanical parameters were calculated from the force-displacements curves (Fig.17):

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

The nominal implant surface area was used to normalize data. This was done since there were small variances in implant height and diameter of the tested implants.

The nominal implant surface was calculated as:

Implant height (mm) x outer implant diameter (mm) x π

Height and diameter was measured twice with a caliber and the average of both values was used to calculate surface area.

Maximum shear strength was defined as the peak on the stress-displacement curve. It represents the maximum stress the implant can tolerate before failure of fixation. Both bone and fibrous tissue can tolerate high stress before failure.

Maximum shear stiffness was calculated as the maximum slope from five consecutive points on the elastic part of the stress-displacement curve. The stiffness reflects the tissue at the interface. Bone has a high stiffness and elastic modulus, while fibrous tissue has a low stiffness and modulus. The maximum energy absorption was calculated as the area under the curve until failure. It represents the needed energy to cause failure of implant fixation. Fibrous tissue can absorb high amounts of energy before failure. Two implants can have the same maximum energy absorption but different stiffness and

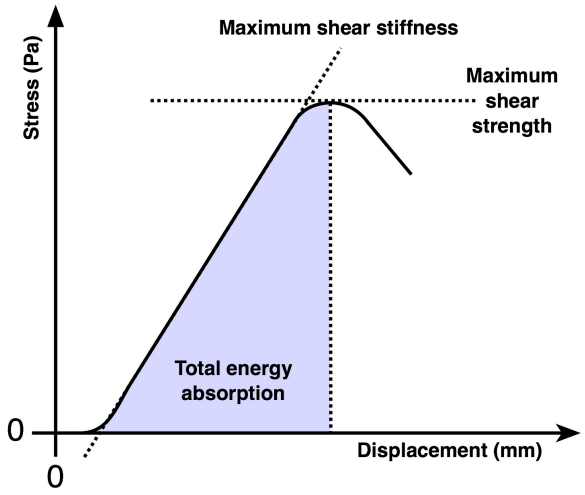


Figure 17. Stress-displacement curve.

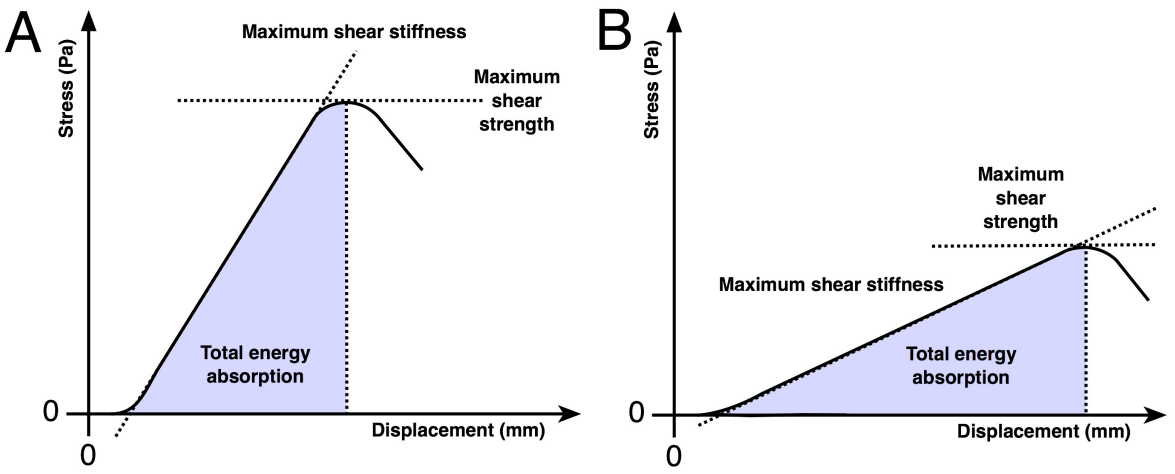


Figure 18. A: Stress-displacement curves from implants osseointegrated (A) or fixated by fibrous tissue (B). Both situations require the same total amount of energy before failure.

strength (Fig. 18).

Reproducibility of the mechanical parameters was not performed since the estimated values were autogenerated from stress-displacement curves.

Clinical interpretation of mechanical testing

Successful long-term survival of a total joint replacement requires stable initial mechanical fixation that allows secondary biological osseointegration to occur [1,2]. If the strain is too high at the interface fibrous tissue will form instead of bone [47]. Furthermore, high strain in form of micromovement can induce bone resorption and fibrous tissue formation [53]. Fibrous tissue will allow transportation of fluid and wear particles from the articulation to the interface [178].

Fixation of an implant can be described by the three parameters discussed in the previous section. In the context of strain resistance, improvement in the maximum shear stiffness seems highly clinical relevant. An osseointegrated implant with high shear stiffness has a high ability to withstand load-inducted micromovements. Translated into a clinical context; during each gait cycle stress will be transferred from the joint prosthesis to the bone bed. The magnitude of prosthetic micromovement at the interface will be determined by stiffness. A joint replacement with a high stiffness has a high ability to withstand everyday load-induced micromovement, remain bony osseointegrated and thereby a high chance for long-term survival (Fig. 19).

It seems likely to assume that stress applied to a joint replacement from everyday use is far from the limit of failure or the maximum shear strength (Fig. 19). It is only in situations such as trauma with high energy applied to the hip, that total disintegration between prosthesis and bone bed occurs. In these situations the bone bed will fracture and the result will

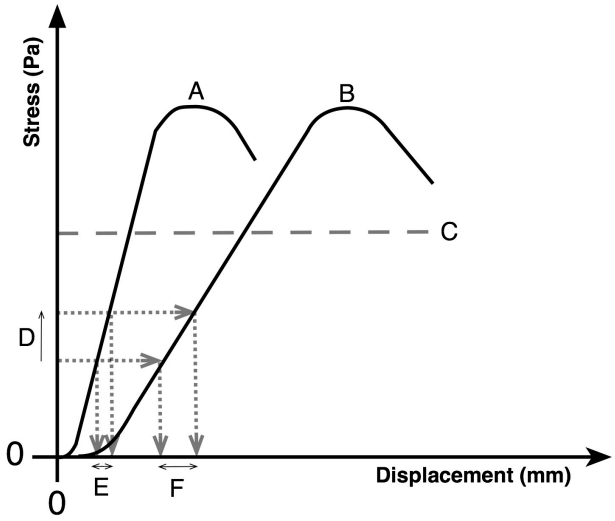


Figure 19. A given stress (D) can result in two different magnitudes of implant displacement (E or F) depending on the stiffness of the bone-implant interface (A or B). Values below C represent everyday stress applied to an implant.

be a peri-prosthetic fracture. Focus on improving stiffness is important in the quest of prolonging implant longevity.

Histomorphometrical analysis

Bone regeneration and implant osseointegration can be quantified with histomorphometrical analysis. Bone-implant specimens from **Study I-VI and VIII-X** were gradually dehydrated in ethanol (70%-99%) containing 0.4 % basic fuchsin, and embedded in methylmethacrylate (MMA). Bone-implant specimens from **Study VII and XI** contained a PMMA implant that would dissolve in MMA. Embedding of these specimens was done in Technovit 7100 (Heraeus Kulzer, Germany) (**Study VII**) or Epoxy (EPOFIX, Struers A/S, Denmark) (**Study XI**). Embedding was done in cylindrical molds that allowed the vertical axis of the implant to be parallel with the long axis of the mold. After random rotation of the implant, four sections were made parallel to the vertical axis of the implant with a hard tissue microtome (KDG-95, MeProTech, The Netherlands)(Fig. 20). The sections were obtained from the central part of the implant and cut with a distance of 0.4 mm. The thickness of each section was 20-30 µm. Sections from **Study I-VI and VIII-XI** were counterstained with 2% Light Green (BDH Laboratory Supplies, Poole, England) for 2 minutes[179]. Penetration of Light Green into the section after two minutes

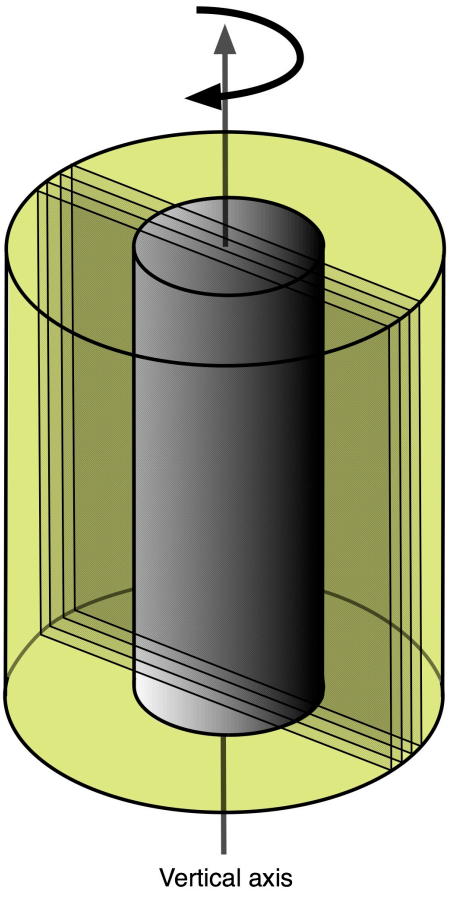


Figure 20. The embedded implant was randomly rotated around the vertical axis before four vertical sections were cut.

was 5-10 µm. Sections from **Study VII** were counterstained with 0.1 % toluidine blue (pH 7) (Sigma-Aldrich, St. Louis, Missouri). All sections were mounted on glass after being counterstained.

Histological evaluation was done using a light microscope (objective x10, ocular x10). Histomorphometrical evaluation was done using stereological software (CAST-Grid, Olympus, Denmark). Fields of vision from the microscope were transferred to a computer. The CAST-Grid software superimposes test probes onto the fields of vision and allows histomorphometrical parameters to be estimated. Osseointegration of implants was quantified as bone-to-implant surface contact. Amount of bone around the implants were quantified as bone volume fractions in predefined regions of interest (ROI).

The different types of tissue were discriminated from each other based on their morphological appearance and staining. Light Green and toluidine blue stained bone green and blue, respectively, made it easy to discriminate from other tissues. Immature newly formed bone was woven and less organized with large osteocyte lacunae. Mature bone was organized with parallel lamellar and small oval osteocyte lacunae orientated parallel to the lamellar. Allograft bone was lamellar bone with spiked borders and empty osteocyte lacunae. Fibrous tissue was stained red and was identified by the presence of its fiber complexes and low cell density. The fibers could either by parallel and high organized as a membrane around the implants or loosely organized. Bone marrow consisted of either empty fat vacuoles or cluster of bone marrow cells. Preparation of specimens and subsequent analysis was done blinded.

Stereological histomorphometry

Estimates of surface and volume fractions in this thesis arise from two-dimensional histological sections. However, implant osseointegration (bone surface fraction) and peri-implant bone density (bone volume fraction) is three-dimensional quantities. Quantification of three-dimensional tissue properties from two-dimensional samples can be associated with bias if done incorrectly. The solution is to use stereological principles when estimating these fractions. Stereology is the three-dimensional interpretation of two-dimensional cross sections. The word "stereology" was coined in 1961 by the Foundation of the International Society of Stereology. In order to avoid bias, three requirements have to be met in the stereological design:

Choosing the correct probe
The first step in obtaining an unbiased stereological estimate is to choose the correct probe. A probe is a geometric shape (e.g. lines or points) that is overlaid the object of interest (e.g. surface or volume) and used to obtain quantitative information about the object. Estimates of first order stereological object (number, length, surface and volume) require that sum of dimension of the probe and object is at least three. Hence, surface (2D object) requires a line (1D object) as probe and

volume (3D) requires a point (0D) as probe[180]. If the sum of dimensions is less then three, then the object of interest could "hide" in the excluded dimension, and therefore not be counted.

Isotropy

The second step in obtaining an unbiased estimate is to ensure that all potential probe-object interactions are of equal probability and that probe-object interactions counted during histomorphometrical analysis are chosen random. This means that the stereological design must take into account that tissue rarely is isotropic (the property of being identical in all directions). An anisotropic tissue will have at least one preferred directed in the coronal, horizontal and/or sagittal plane. The anisotropy will imply that some probe-object interactions are more likely to by counted than others. In other word, the object size, shape, orientation and distribution in space must not affect the estimate. If this is not fulfilled, then bias can occur. The problem with anisotropy can be overcome by ensuring that either the probe or object is isotropic orientated in all three planes (coronal, horizontal and sagittal) [180,181]. Stereological software using mathematical principles can in some cases make a probe isotropic in one plane (the plane of the section). The used sectioning method can make the object of interest isotropic in all planes. This can be achieved if all planes are chosen random to their respective axis. Sections that are isotropic in all three planes are known as Independent Uniform Random (IUR) sections [182]. The vertical section method used in this thesis has two random planes [183].

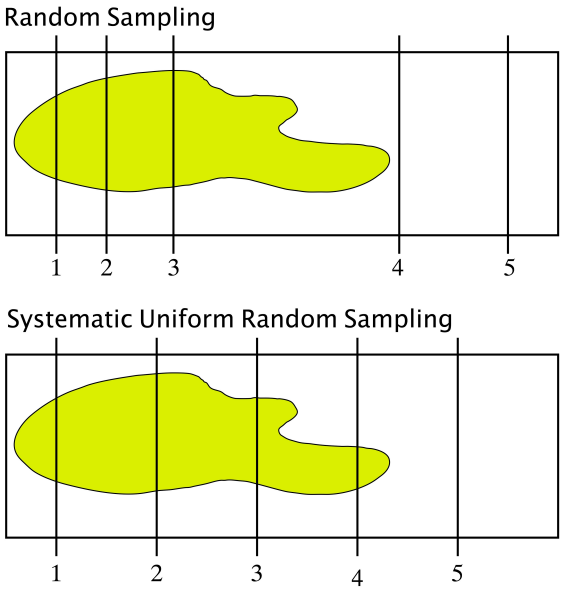


Figure 21. Simple random sampling: All sections are cut random. Systemic Uniform Random Sampling: The structure is cut with uniform constant intervals and random start for section 1.

Systematic Uniform Random Sampling

The third and last step to avoid bias is to ensure that the histological sections are representative of the organ/material of interest – in this thesis the implant and surrounding tissue. The histological sections have to be chosen random. This can be achieved by simple random sampling [180]. The problem with simple random sampling is that many sections have to be included to obtain an acceptable precision, since the object of interest rarely is distributed evenly throughout the organ. (Fig. 21). Systematic uniform random sampling can overcome this problem [184]. With this method, the material is sliced in regular uniform intervals, but the first cut is chosen randomly (Fig. 21). Systemic uniform random sampling from the central

part of the implants was used in all studies in this thesis.

Considerations on stereological designs

In this thesis two histomorphometrical parameters were estimated: bone-to-implant surface contact and bone volume density. This implies that two different probes must be used. In order to avoid bias, the stereological design in this thesis must therefore ensure anisotropy and systematic uniform random sampling for both probes.

Estimating surface – The Vertical Section method

Unbiased surface estimates can be obtained using the vertical section method described by Baddeley et al [183]. The method has four requirements (Fig. 22): 1) A vertical axis is defined. In this thesis the long axis of the implants were used. 2) The specimen is rotated around the vertical axis with random stop. 3) Sections are cut parallel to the vertical axis. Ideally, the sections should be selected by Systematic Uniform Random Sampling (SURS) from the entire specimen. In this thesis the sections were selected by SURS from the central part of the implant. 4) In each section the direction of the vertical axis is identified and orientation of test probe lines are weighted proportionally to the sine of a random angle originating from the vertical axis. Cycloid test probes can be used instead of sine weighted lines. Requirement 1,2 and 4 ensures an isotropic design in all three dimensions. Requirement 3 ensures SURS.

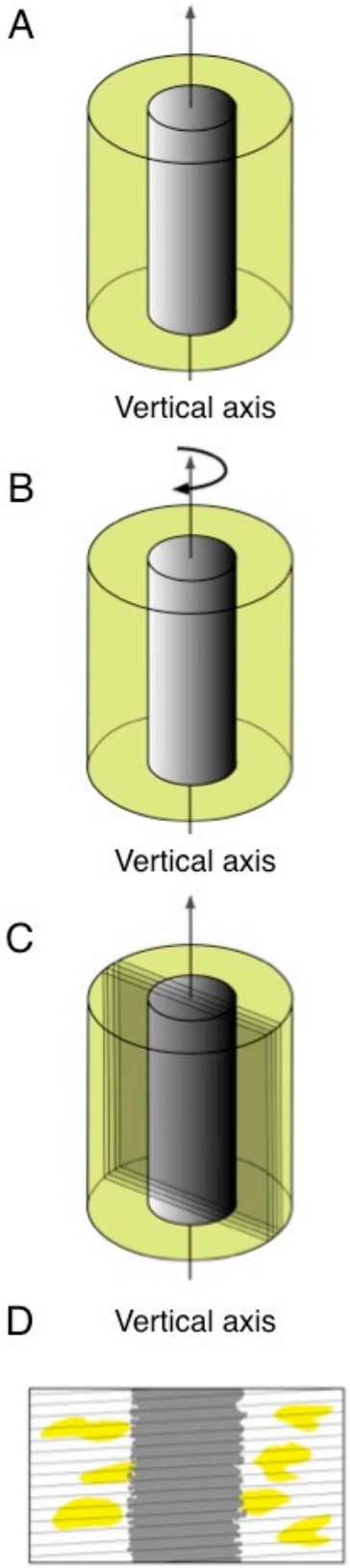


Figure 22. A: Vertical axis identified. B: Random rotation around vertical axis. C: Section cut parallel to vertical axis. D: Sine weighted lines used.

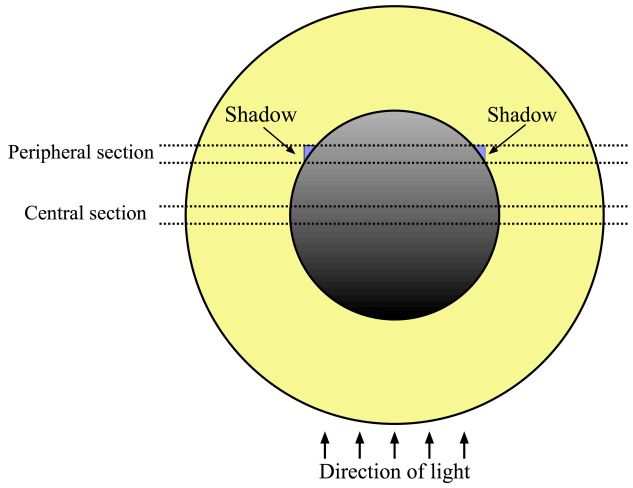


Figure 23. Shadow effect. A peripheral section will cast a shadow. Only a central section will be without a shadow

The use of a cylindrical implant in the vertical sections introduces a risk of bias in the surface estimates. This is due to the shadow effect of the opaque implant. The shadow effect increases with the distance from the center of the implant (Fig. 23). Clustering the systemic uniform random sampling of section to the central portion of the implant will reduce potential bias from the shadow effect. However, this makes the sections less representative of the entire specimen, since sampling is not the whole specimen but only a cluster (Fig. 24). A trade off has to be made between reducing the shadow effect and ensuring representative sections. Reducing the shadow effect was chosen in this thesis as described by Balatsouka [185].

Estimating volume – The Point-Counting Estimator

The point-counting estimator can obtain unbiased estimates of volume fractions. A point is a 0D structure and does therefore not have any preferred spatial direction. A point is isotropic by nature. This means that no special considerations have to be done on ensuring isotropy when preparing the sections [186]. It is however important to ensure that the sections made for histomorphometrical volume estimates are representative of the region of interest (ROI). This can be achieved by SURS from the entire ROI [180,187].

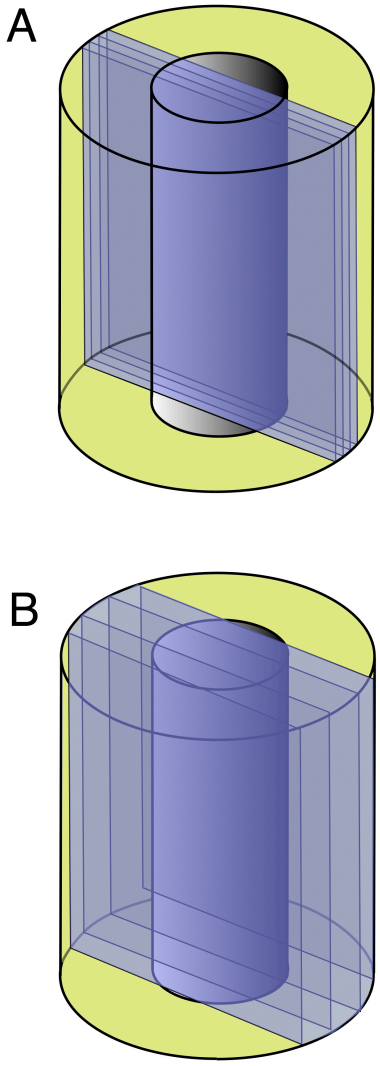


Figure 24. A: Small section sampling coverage with reduced shadow effect. B: Large section sampling coverage with relative large shadow effect.

Figure 25 shows the complete ROI for volume fraction estimates in this thesis. The ROI covers a concentric space around the implant. The inner border of the ROI was defined as the implant surface. The outer border of the ROI radiated 500-2500 µm from the implant surface. The exact distance between the inner and outer border varied amongst the studies included in this thesis. Given the roughness of the implant surface, an approximated inner surface border line had to be defined. The surface line was defined as described by Bass as a line between the inner most porosity and outer most spike [188].

The systematic uniform random sampling (SURS) of sections from the central part of the specimen containing the implant and not from the entire ROI implies that not all tissue in the ROI have an equal chance of being sampled (Fig. 25). This will introduce a bias, if tissue is not homogeneously distributed with the ROI. Despite the risk of bias, sampling from the central portion of the implant was chosen in order to reduce the impact of *section offset bias*.

Section offset bias occurs when a predefined sampling area is applied to a section that is not coaxial with the central axis of the implant (Fig. 25). The fraction of the ROI covered by the sampling area decreases with the distance between the implant center and section offset. Assuming an implant diameter of 6 mm, maximum section offset of 1.5 mm and a outer border of the ROI 2 mm from the implant surface, then the minimum fraction covered by the sampling area can be calculated to 93%. Bone regenerates from the peripheral zone of the ROI and towards the implant surface. Due to section offset bias, peripheral sections have a higher risk of underestimation volume fractions of new bone compared to central coaxial sections. A tradeoff between low impact of the section offset bias and high degree of

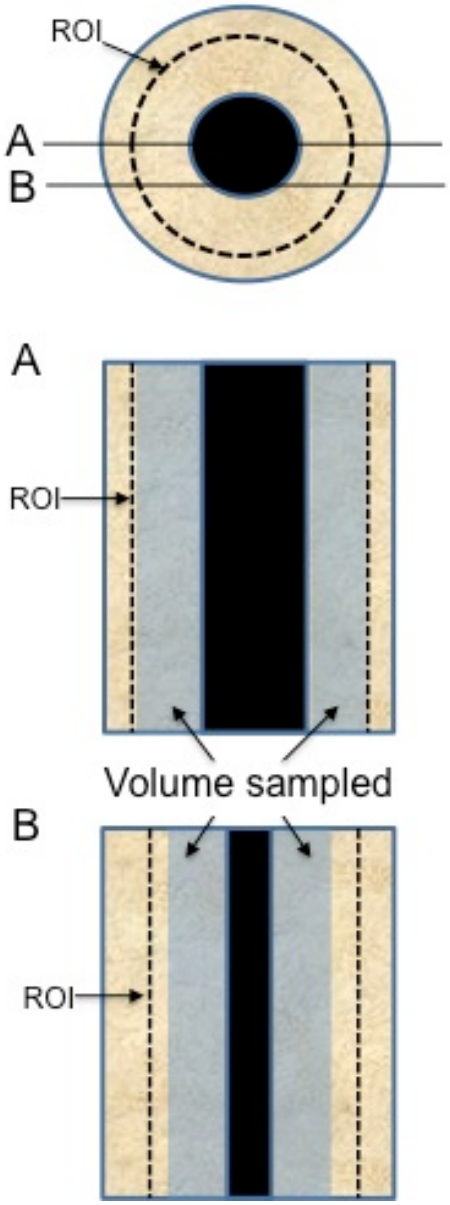


Figure 25. Section offset bias. Dotted line represent outer border of Region of Interest (ROI). The volume sampled and ROI will coincide in a section cut through the center of the implant (A). In a peripheral section the volume sampled will only cover a portion of the ROI.

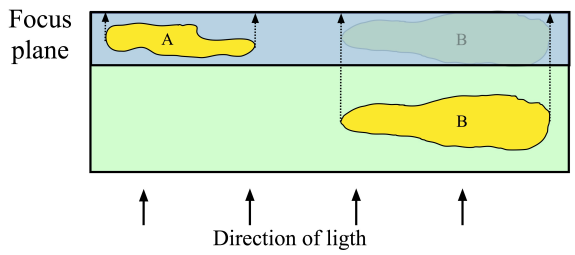


Figure 26. Holmes effect. Opaque tissue deep in the section will be over-projected into the focus plane. This will cause an overestimation.

sampling from the entire ROI had to be made. A high degree of coverage of the ROI was chosen at the cost of reduced sampling coverage.

The sections used in this thesis to estimated volume fractions were 20-30 µm thick. This means that opaque tissue such a bone can be over projected to the focus plan and thereby overestimated (Fig. 26). This is known as the *Holmes effect* [189]. The Holmes effect was reduced in the used design by surface staining the sections and restricting sampling to surface stained tissue. The penetration of the surface staining was 5-10 µm. Bias due to the Holmes effect can also be introduced when estimation implant surface fractions of bone.

Volume fractions estimates are relative to a reference volume. If this reference volume shrinks relative to the tissue of interest during preparation, then overestimation of the given tissue will occur. The phenomenon is known as the *reference trap* [189,190]. Both implants and bone are hard materials with little probability of affecting the reference trap during preparation.

Efficiency of sampling

The purpose of histomorphometrical analysis was to investigate the effect of local bisphosphonate treatment on bone regeneration and preservation at tissue level. The used test animals and histomorphometrical methods add variance to the obtained dataset and introduce the risk of type 1 and 2 errors when the treatment groups are compared statistically. This relationship can be described as[188,191]:

$$CV_{obs}^2 = CV_{bio}^2 + CV_{met}^2$$

CV = Coefficient of Variance
Obs = Observed
Bio = Biological
Met = Methodological

Methodological variance from the histomorphometrical sampling can be subdivided into variance from different sections within each implant, variance among the two sides of each section, variance from field of view, and variance from probe position and orientation [192]. Increasing the sampling intensity at all sublevels can increase the precision of the histomorphometrical method.

Histomorphometrical analysis is time consuming. Increasing the methodological precision will only affect the observed variance if the contribution from the biological variance is relative low. The goal is therefore not to have a low absolute methodological variance, but to keep it low compared to the observed variance. Gundersen et al. recommend the following

relationship between the methodological and observed variance [184]:

$$0.2 < \frac{\mathrm{CV_{met}}^2}{\mathrm{CV_{obs}}^2} < 0.5$$

Overgaard et al. have previously in five humans investigated the relationship between the biological and methodological variance [192]. They found that most of the observed variance came from the biological variance. Implants in this thesis were evaluated with four sections and a probe sampling intensity of 100-200 hits pr. object of interest [184,192].

Reproducibility

Intra-observer variation on histomorphometrical estimates was calculated from double measurements of randomly selected implants from all treatment groups. The period between the two measurements was minimum one month. Intra-observer variation was calculated as coefficient of variation (CV):

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

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

	New bone	Lamellar bone	Marrow
Surface	16%	48%	9%
Volume	6%	2%	0.2%

CV in percent

Table 4: Reproducibility - Model of failed osseointegration

	New bone	Lamellar bone	Fibrous tissue
Surface	N/A	2%	1%
Volume	N/A	4%	1%

CV in percent

Table 5: Reproducibility - Pressfit model

	New bone	Lamellar bone	Marrow
Surface	17%	7%	9%
Volume	5%	3%	5%

CV in percent

The relative high CV values are caused by relative low fractions of the respective tissue. Table 2 and 3 are reproduced from my PhD thesis.

Statistical analysis

Statistical analysis was done using Intercooled Stata 9.0 (Stata Inc. College Station, TX, USA). Student´s t-test for

paired data was used to test for differences for studies with two treatments groups and with normally distributed variables. Some dataset had to be log-transformed before it became normally distributed. Wilcoxon Signed Rank test was used for studies with two treatment groups but not normally distributed variables. ANOVA or Friedman´s test was used to test for differences in studies with four treatment groups and normally or not normally distributed variables, respectively. Dataset that passed ANOVA or Friedman´s test was subsequently analyses with Student´s t-test or Wilcoxon Signed Rank t-test, respective. Two-tailed *p*-values below 0.05 were considered statistically significant.

A detailed description of the statistical analysis used for the studies included in this thesis can be found in the respective articles.

Improving implant fixation

Bone compaction and bisphosphonates (Study I-II and IV)

It has previously been shown that bone compaction can increase fixation of experimental implants[28–30,36]. Bone compaction is a surgical technique that prepares the bone bed for implantation. It compacts the bone bed and creates a dense zone of bone autograft *in situ*. Furthermore, it places the implant in extreme press-fit since the compacted bone has a *spring back effect* [26]. The purpose of **Study I-II and IV** was to investigate whether preservation of the compacted bone with bisphosphonates would further improve implant fixation. This was tested with different observations periods, under different loading conditions and implant surface coatings (Table 6).

Biomechanical results

Local treatment with alendronate was able to increase the mechanical fixation of non-weight bearing implants after 12 weeks of observation **(Study II and IV)**(Table 7). No effect on mechanical implant fixation of local alendronate treatment was found on weight bearing implants after 4 weeks of observation **(Study I)**.

Table 6: Bone Compaction - Overview of study design

Study	*Model*	*Treatment groups*	*Weigth-bearing*	*Implant coating*	*Observation period*
I	Compaction, Femur	Alendronate / saline	Yes	Ti	4 weeks
II	Compaction, Tibia	Alendronate / saline	No	Ti	12 weeks
IV	Compaction, Tibia	Alendronate / saline	No	HA	12 weeks

Ti = Titanium, HA= hydroxyapatite

Table 7: Biomechanical results

	Max shear strength, MPa	Max shear stiffness, MPa/mm	Total energy absorption, kJ/m^2
Study I			
Alendronate	7.1 (5.0-7.9)	33.1 (26.1-38.2)	1.1 (0.8-1.2)
Control	6.6 (5.7-8.0)	30.6 (27.7-39.7)	1.0 (0.7-1.1)
Study II			
Alendronate	6.7 (4.9;8.4)*	23.6 (18.2;29.0)*	1.7 (1.2;2.1)*
Control	3.0 (1.8;4.2)	12.3 (7.4;17.0)	1.0 (0.5;1.4)
Study IV			
Alendronate	2.9 (2.2;3.6)*	25.2 (20.0;30.7)*	0.5 (0.3;0.7)
Control	1.6 (1.0;2.2)	10.0 (6.2;15.6)	0.3 (0.2;0.4)

Study I: Median and ranges, Study II and IV: Mean and 95%CI. *p<0.05 when compared with control.

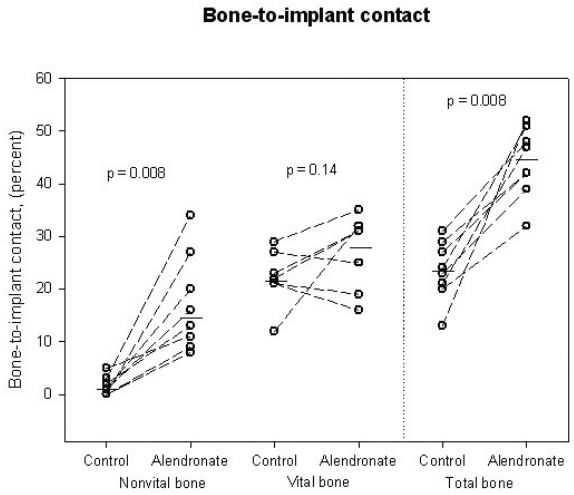


Figure 27. Study I. Bone-to-implant contact. Paired data connected by a dotted line.

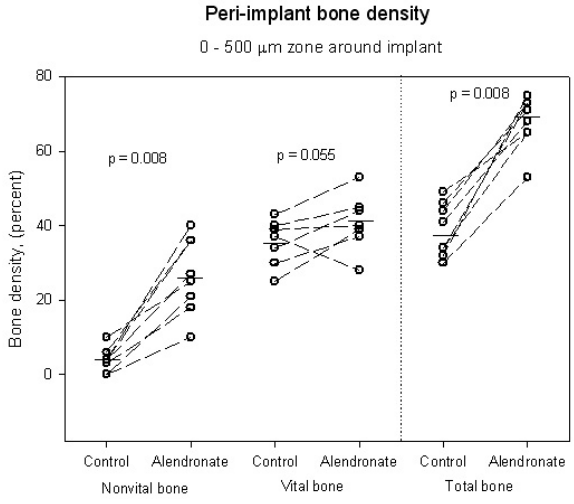


Figure 28. Study I. Volume fraction of bone. Paired data connected be a dotted line.

Histomorphometrical results

The primary effect of local alendronate treatment was an increase in the volume fraction of bone around the implants **(Study I-II and IV)**(Fig. 27-30). **Study I and II** were able to demonstrate an increase in bone-to-implant contact. No effect on bone-to-implant contact was found around the hydroxyapatite coated implants in **Study IV**. Local alendronate

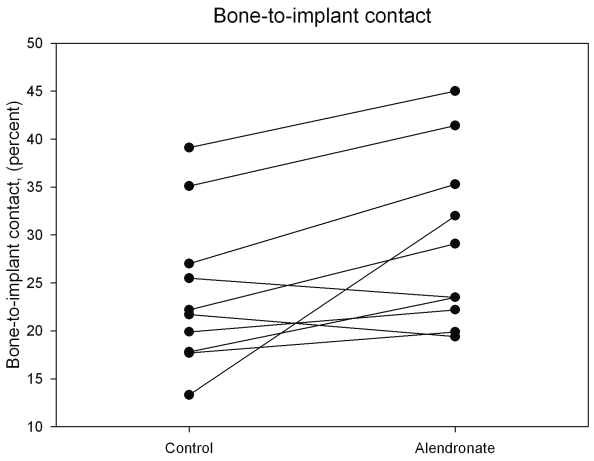


Figure 29. Study II. Bone-to-implant contact. Paired data connected by line, p = 0.043

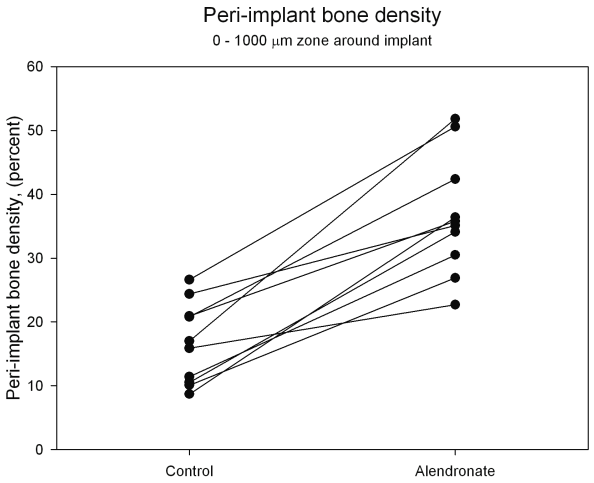


Figure 30. Study II. Bone density in a 0-1000 µm zone around implants. Paired data are connected by line, p<0.0001.

treatment was able to increase new bone formation and preserve old lamellar bone.

Histology

Figure 31 shows a representative example of the most striking histological difference found in **Study I-II and IV**. Figure 31 shows a 1 mm dense zone of bone around the implant from the alendronate group. No histological differences were observed further away from the implant surface. No

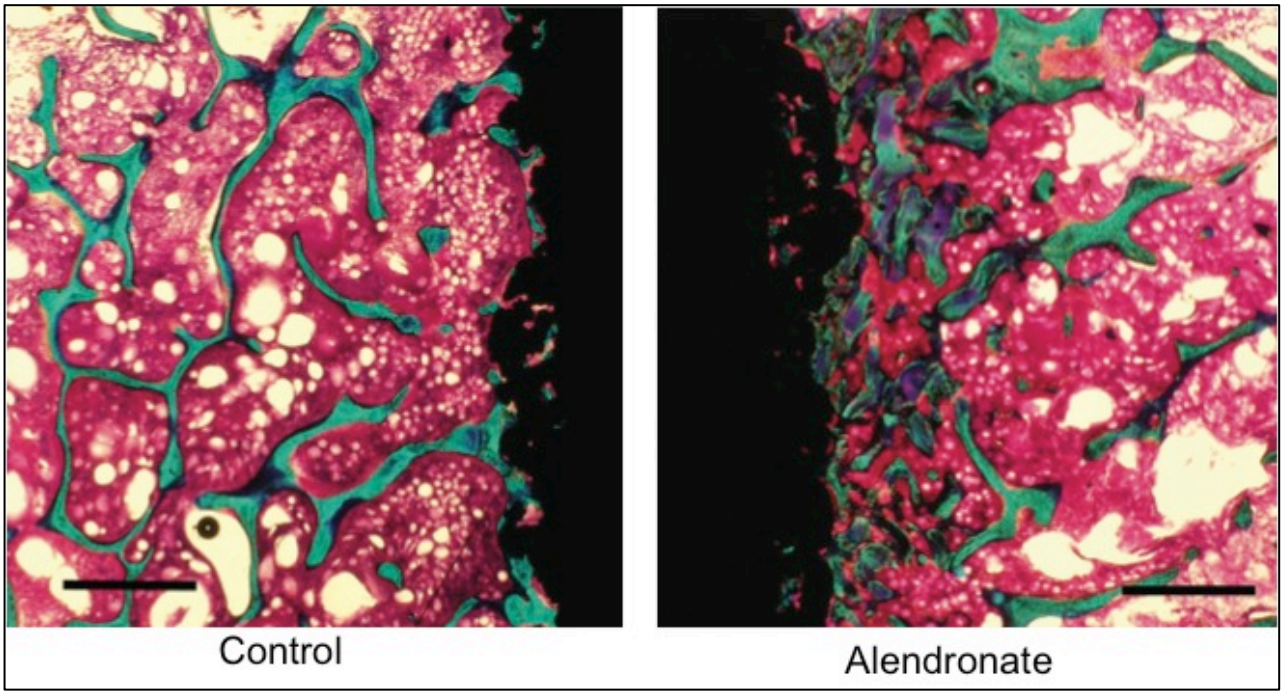


Figure 31. Representative histological samples from the same animal (Study II). Implant appear as black, marrow as red, and bone as green. Control implant and alendronate implant. Note the increased amount of bone around and on the alendronate implant (right). Bar = 1.0 mm.

delamination of the hydroxyapatite coating was observed in **Study IV**.

Discussion of findings

Bisphosphonates have strong affinity for bone[98]. When locally added to bone most of the bisphosphonate will adsorb to exposed bone surfaces. The remaining will stay unbound in solution between the trabeculae. A too high concentration of bisphosphonate might impair osteoclast and osteoblast function. The dose used in **Study I-II and IV** was based on previous studies from the literature demonstrating an effect of local alendronate treatment [132,193]. During surgery of **Study I**, bleeding was observed from the bone marrow and through the implant cavity. This bleeding could potentially wash away the alendronate. The concentration of alendronate was doubled from 1 mg/mL in **Study I** to 2mg/mL in **Study II and IV.** This was done in order to increase the amount of alendronate retained in the bone bed. Unbound alendronate was not rinsed away. The results from **Study III** shows that alendronate in a solution of 2 mg/mL can impair bone formation within allograft. The bleeding from the bone marrow might have washed away the unbound potential toxic alendronate in **Study I-II and IV** and prevented a deleterious effect on implant fixation.

Alendronate increased mechanical fixation of the non-weight bearing implant in **Study II and IV**. A likely explanation could be the increased amount of bone around the implants. These results are supported by clinical findings showing that both local and systemic bisphosphonate treatment

reduces implant migration measured by RSA [146–148,194]. However, other factor than peri-implant bone density affects implant fixation. Study I is an example of this. In this study an increased bone density was observed without any effect on mechanical implant fixation.

Common for **Study I-II and IV** was the preservation of lamellar bone. This finding was expected due to the anti-resorptive effects of alendronate[97,195]. Several experimental studies have found that both systemic and local bisphosphonate treatment can preserve bone [106,107,109,118,132,142,196]. Furthermore, the bone preservative effect of bisphosphonate was also found in clinical studies [149,197].

An increased amount of new bone was found around the implant in **Study IV**. One explanation for this could be that alendronate preserves the autograft created *in situ* and thereby prolongs the osteoconductive properties of the autograft. Another explanation could be that alendronate slows down remodeling of new bone into lamellar bone and thereby shifts the balance towards more new bone after 12 weeks[198]. A third explanation of the increased amount of new bone could be a direct stimulatory effect of alendronate on bone formation [101,199].

Implant osseointegration is a temporal process. With only one observation period it is difficult to conclude which stage in osseointegration is evaluated. An increased mechanical implant fixation was observed in **Study II and IV** after 12 weeks of observation, but not in **Study I** after 4 weeks of observation. An explanation for the lack of increased implant fixation in **Study I** could be that formation of new bone in the used model of bone compaction requires more time than 4 weeks. This is supported by the finding by Kold et al. who demonstrated a mechanical effect of bone compaction on implant fixation after 0 weeks of observation, but not at 4 weeks [28].

Another explanation for the observed effect in mechanical implant fixation between **Study I** and **Study II + IV** could be the difference in weight bearing conditions. **Study I** included weight-bearing implants while **Study II and IV** included non-weight bearing implants. Implant osseointegration is under influence of loading[43,47,80]. It could be that weight bearing positively stimulated new bone formation in **Study I** thus making it difficult for the alendronate treatment to further increase implant fixation after only 4 weeks of observation.

Hydroxyapatite is a bioactive coating with osteoconductive properties [62,200]. Experimental studies have shown that HA-coated implants achieves increased osseointegration compared to Ti-coated implants [62]. Other experimental studies have found that bisphosphonate can increase osseointegration of HA-coated implants [108,118]. The rationale behind **Study IV** was to test whether alendronate was able to further increase osseointegration of HA-coated implants inserted with bone compaction. However, no difference in new or lamellar bone in contact with the HA-coated implant surface

Table 8 : Bone Graft studies - Overview of study design

Study	*Model*	*Treatment groups*	*Type of graft*	*Implant coating*	*Observation period*
III	2.5 mm Gap, Humerus	A: Alendronate, 4 weeks B: Control, 4 weeks A: Alendronate, 12 weeks B: Control, 12 weeks	Morselized allograft	Ti	4 weeks 12 weeks
V	2.5 mm Gap, Humerus	A . Control B. 0.005 mg Zol/mL C. 0.05 mg Zol /mL D. 0.5 mg Zol / mL	Morselized allograft	Ti	4 weeks
VI	2.5 mm Gap, Tibia	A. Control B. Zoledronate	TCP granules	Ti	12 weeks
X	2.5 mm Gap, Femur	A. Control B. BMP-2 C. Zoledronate D. BMP-2 + Zol	Morselized allograft	HA	4 weeks

was found in Study **IV**. One explanation for this could be that the combined effect of HA-coating and bone compaction leaves little room for improvement in osseointegration. The increased mechanical fixation in **Study IV** can be described by the effect of alendronate on the peri-implant bone bed.

Bone graft and bisphosphonates (Study III, V-VI and X)

Bone grafts are used in situations with insufficient bone bed and reduced bone stock [74]. The purpose of the graft material is to add primary mechanical stability and facilitate secondary biological fixation. Experimental rodent studies have shown that bisphosphonate treatment can preserve bone allograft and increase amount of new bone [131,132]. This is reflected in a clinical study where local ibandronate treatment preserved morselized allograft [152].

Study III, V-VI and X evaluated the effect of bisphosphonate on grafted implant fixation in different settings (Table 8). Common for all studies was soaking of bone grafts in bisphosphonates followed by impacting it around an implant. The purpose of **Study III** was to evaluate the effect of local bisphosphonate treatment on morselized allograft and implant fixation after different observation periods. **Study III** showed that soaking allograft in bisphosphonate impaired implant fixation. This was unexpected. A dose-response study was needed. **Study V** investigated the dose-response between bisphosphonate and implant fixation. BMP-2 can increase

Table 9: Study III - Biomechanical results

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

Mean and 95%CI. * p < 0.05 when compared with control

Table 10: Study V – Biomechanical results

Groups	Max shear strength, MPa	Max shear stiffness, MPa/mm	Total energy absorption, kJ/m^2
Control	1.9 (1.2-2.7)	8.9 (5.6-12.2)	0.4 (0.2-0.6)
0.005 mg Zol/mL	2.6 (1.4-3.7)	10.5 (5.6-15.4)	0.7 (0.4-1.0)
0.05 mg Zol/mL	1.0 (0.6-1.5)*	4.0 (2.1-5.8)*	0.4 (0.2-0.6)
0.5 mg Zol/mL	0.4 (0.3-0.4)*†	0.8 (0.6-1.1)*†	0.3 (0.2-0.4)‡

Mean and 95%CI;*p < 0.05 when compared with control group, †p < 0.05 when compared with middle-dose,‡p < 0.05 when compared to the low dose

Table 11: Study X – Biomechanical results

Groups	Max shear strength, MPa	Max shear stiffness, MPa/mm	Total energy absorption, kJ/m^2
Control	5.4 (2.2)	22.3 (8.0)	1.22 (0.53)
BMP-2	4.2 (1.3)	15.2 (6.1)	1.09 (0.55)
Zoledronate	7.6 (2.5)	34.5 (6.1)	1.26 (0.53)
BMP-2 + Zoledronate	5.1 (2.0)	21.0 (9.6)	1.28 (0.58)
ANOVA	p <0.001	p<0.001	p=0.794

Mean and SD

Table 12: Study VI - Biomechanical results

Groups	Max shear strength, MPa	Max shear stiffness, MPa/mm	Total energy absorption, kJ/m^2
Zoledronate	2.45 (0.70;2.22)	17.09 (3.95;30.23)*	0.38 (0.12;0.65)
Control	1.00 (0.44;1.56)	5.32 (1.53;9.11)	0.21 (0.13;0.29)

Mean and 95%CI. * $p < 0.05$ when compared with control

new bone formation and graft resorption[201]. **Study X** investigated whether local bisphosphonate treatment could counteract the increased graft resorption while maintaining or increasing new bone formation. The purpose of **Study VI** was to investigate whether soaking a bone graft substitute such as TCP granules would increase new bone formation.

Biomechanical results

Study III found a dramatically impairment of mechanical implant fixation (Table 9). A dose-response relationship was found in **Study V** after 4 weeks of observation. The low dose of zoledronate resulted in superior mechanical implant fixation compared to middle and high dose (Table 10). The effect of soaking allograft in the low dose of zoledronate was reproduced in **Study X**. However, **Study X** failed to demonstrate that zoledronate could counteract the effects of BMP-2, since the combination of zoledronate and BMP-2 impaired implant fixation (Table 11). **Study VI** showed that soaking TCP granules in zoledronate could improve mechanical implant fixation (Table 12).

Histomorphometrical results

The local alendronate treatment in **Study III** virtually blocked new bone formation and allograft resorption after both 4 and 12 weeks of observation (Figure 32 and 33).

In Study V, a dose dependent difference in the amount of new bone was found. The

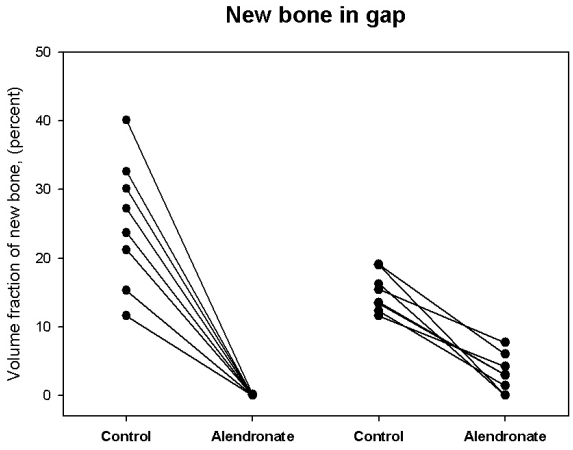


Figure 32. Study III. Volume fraction of new bone in gap. Paired data connected by line.

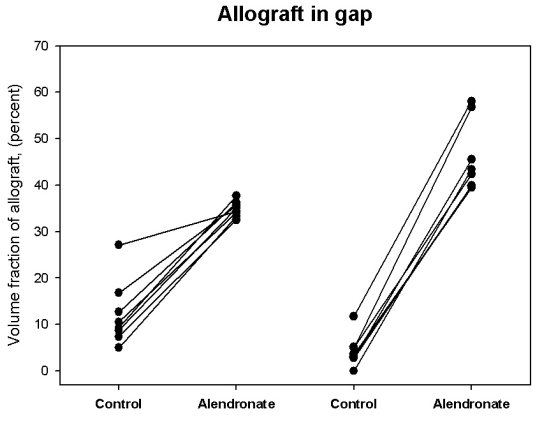


Figure 33. Study III. Volume fraction of allograft in gap. Paired data connected by line.

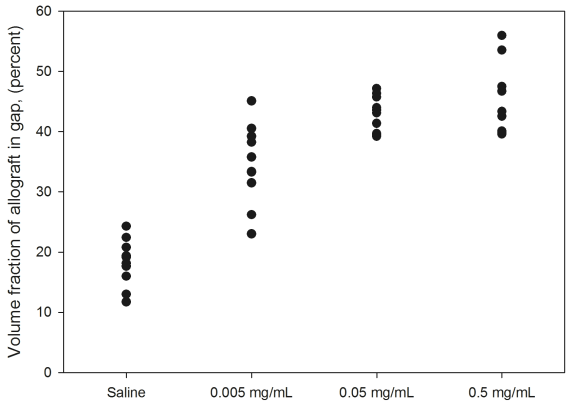


Figure 34. Study V Volume fraction of allograft in gap.

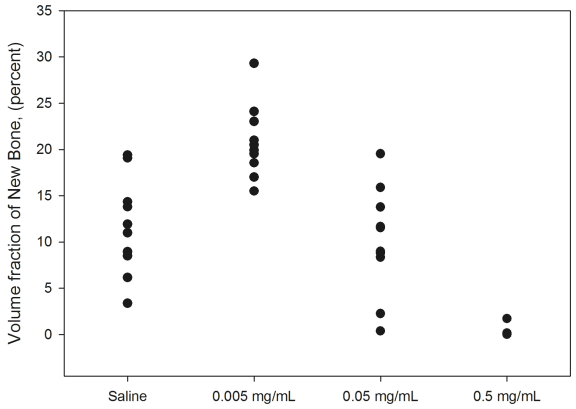


Figure 35. Study V. Volume fraction of new bone in gap

low zoledronate dose gave the highest amount of new bone. The highest dose of zoledronate blocked new bone formation (Fig. 35). Increasing the concentration of zoledronate resulted in increased preservation of the allograft. A ceiling effect in graft preservation was observed from the middle- to the high-dose of zoledronate (Fig. 34).

The increased mechanical implant fixation in **Study VI** obtained by soaking TCP granules in zoledronate was not reflected in the histomorphometrical results. No difference in new bone or amount of TCP was observed between control and intervention group.

Study X reproduced the results of **Study V** with respect to allograft preservation. Zoledronate alone was able to preserve allograft, but failed to counteract the effects of BMP-2. Although the combination of BMP-2 and zoledronate impaired mechanical implant fixation, no difference in new bone formation was observed.

Histology

A general histological picture of allograft soaked in too high doses of bisphosphonate was the presence of allograft chips and the absence of new bone formation (Fig. 36). For allograft soaked in optimized doses of bisphosphonate, the histological finding consisted of allograft chips encapsulated of new bone (Fig. 37).

Discussion of findings

Several studies have shown that systemic and local bisphosphonate treatment can preserve allograft and amount of newly formed bone [131,132,134,198,202]. **Study III** was one of the first studies to show that soaking morselized allograft in alendronate could impair new bone formation. The findings were unexpected. The allograft in **Study III** was soaked in 5 mL bisphosphonate solution containing 2 mg alendronate pr. mL saline. The same concentration of alendronate resulted in increased mechanical implant fixation, bone preservation and increased amount of new bone in **Study II and IV**. In **Study III**, the concentration of 2 mg alendronate pr.

mL saline resulted in the opposite. The impairment of new bone formation has been reproduced in the same model with pamidronate instead of alendronate [136]. Bisphosphonate can be retained in allograft in two ways. One part of the bisphosphonate will adsorb to the bone surface and be pharmacologically inactive until released by osteoclasts. A second part of the bisphosphonate will remain in will be in solution between the allograft chips. The unbound bisphosphonate has the potential to affect both osteoclasts and osteoblasts. Agholme and Aspenberg have

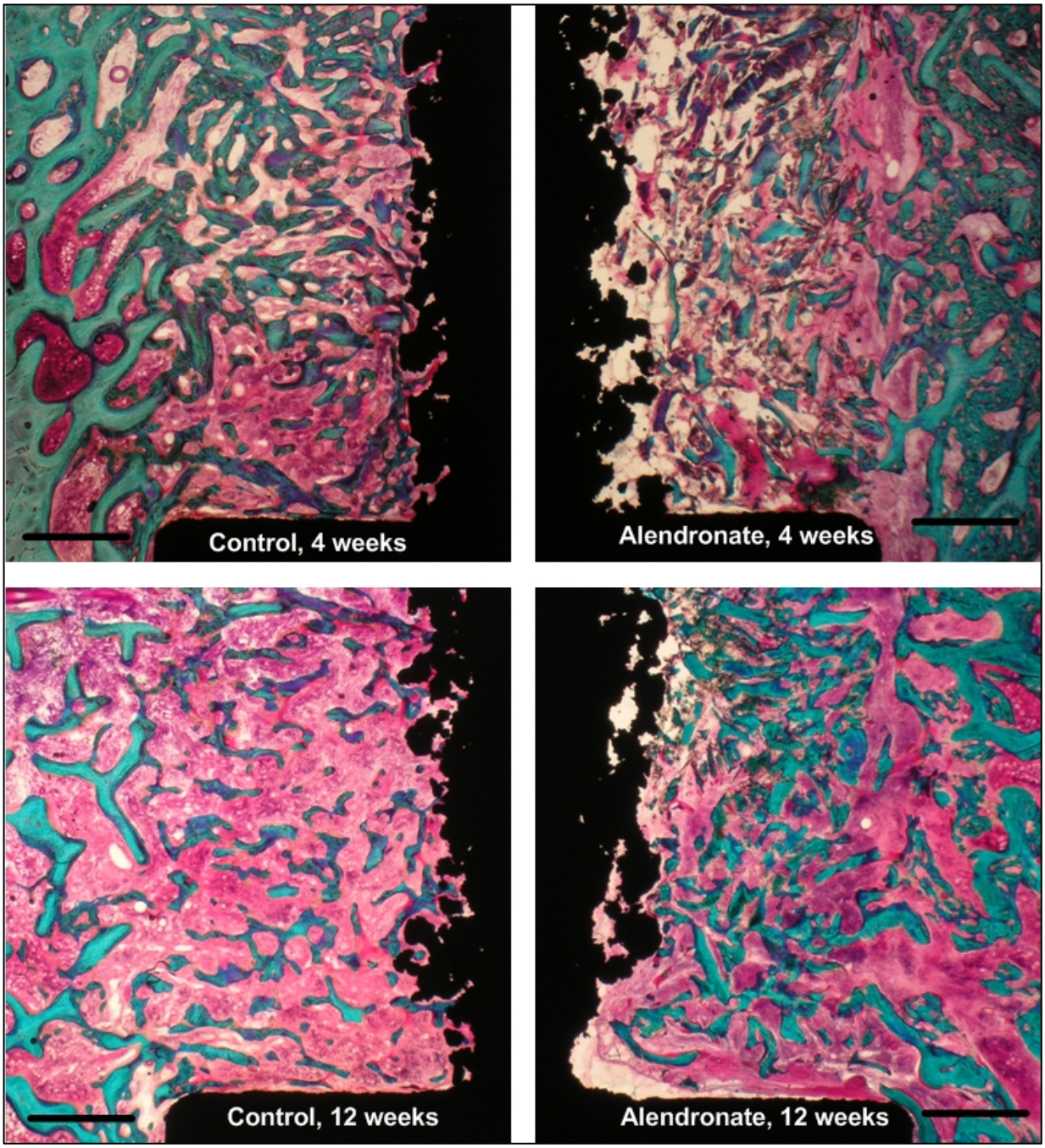


Figure 36. Representative histological samples from the same animal (Study III). Increased amount of bony grains are seen in the gap around the alendronate implants compared with the control implants. The allograft around the control implants seems to be more remodeled and connected by new bone than the allograft around the alendronate implants. Note the qualitatively unaffected bone outside the gap around all implants. Bar = 1.0 mm.

estimated that the largest source of bisphosphonate within allograft comes from the unbound bisphosphonate in solution [203]. Rinsing the allograft reduces the amount of unbound bisphosphonate. The allograft from **Study III** was not rinsed. This meant that unbound alendronate potentially could impair osteoblast function. However, the findings by Agholm and Belfrage do not support this explanation [134,203]. They have found that omission of rinsing did not affect new bone formation.

Another explanation for the impaired new bone formation in **Study III** could be the allograft density. It has previously been shown that impacting allograft impairs its osteoconductive properties [78]. A too dense allograft requires the coupled effect of the osteoclast and osteoblast in creeping substitution to be incorporated by new bone. A less dense allograft can be incorporated by new bone formed by intramembranous ossification. The process is independent of osteoclasts. The un-impacted volume of the allograft used in **Study III** was 1 mL. This amount of allograft was impacted into a volume of 0.7 mL. It could be that the allograft density in **Study III** requires osteoclasts to resorb bone before osteoblasts can form new bone. The impairment of new bone formation in **Study III** was thereby due to the impaired osteoclast function. This is supported by a study of Jeppsson et al. who found that local clodronate reduced the ingrowth of new bone into extremely impacted allograft [202]. The results of **Study V and X** disprove the explanation of graft density being the cause for impaired bone formation in **Study III**. **Study III, V and X** used the same degree of graft impacted. The main differences between the studies were the type of bisphosphonate (alendronate in **Study III** and zoledronate in **Study V and X**), rinsing (no rinsing in **Study III**) and concentration of bisphosphonate. This indicates that the impaired bone formation in **Study III** might be due to the used concentration.

Study V was one of the first studies to demonstrate a dose-response relationship between bisphosphonate concentration and formation of new bone within allograft. A similar dose-response has been found by Mathijssen et al[204]. Belfrage et al. found that the effective retained dose of bisphosphonate is affected by the soaking time[133]. The purpose of soaking allograft in bisphosphonate is to prolong the graft remodeling and retain the load-bearing capacity while allowing new formation within the graft. **Study V** shows that, increasing concentrations of bisphosphonate results in increased preservation of the graft, but reduced new bone formation. The goal was to find the optimal concentration of bisphosphonate that both preserves allograft and allows new bone formation. In **Study V** this concentration was 0.005 mg zoledronate pr. mL. Higher concentrations of zoledronate seem to be increasingly toxic for the osteoclasts.

Study V and X showed that soaking allograft in zoledronate enhances the mechanical implant fixation. A likely explanation for this increase in fixation is the increased amount of new bone. This is

similar to the results obtained by combining bone compaction and bisphosphonate in **Study II and IV**.

Local zoledronate treatment in **Study V and X** was able to increase the amount of new bone. This is supported by other studies [132,133,202]. The increased

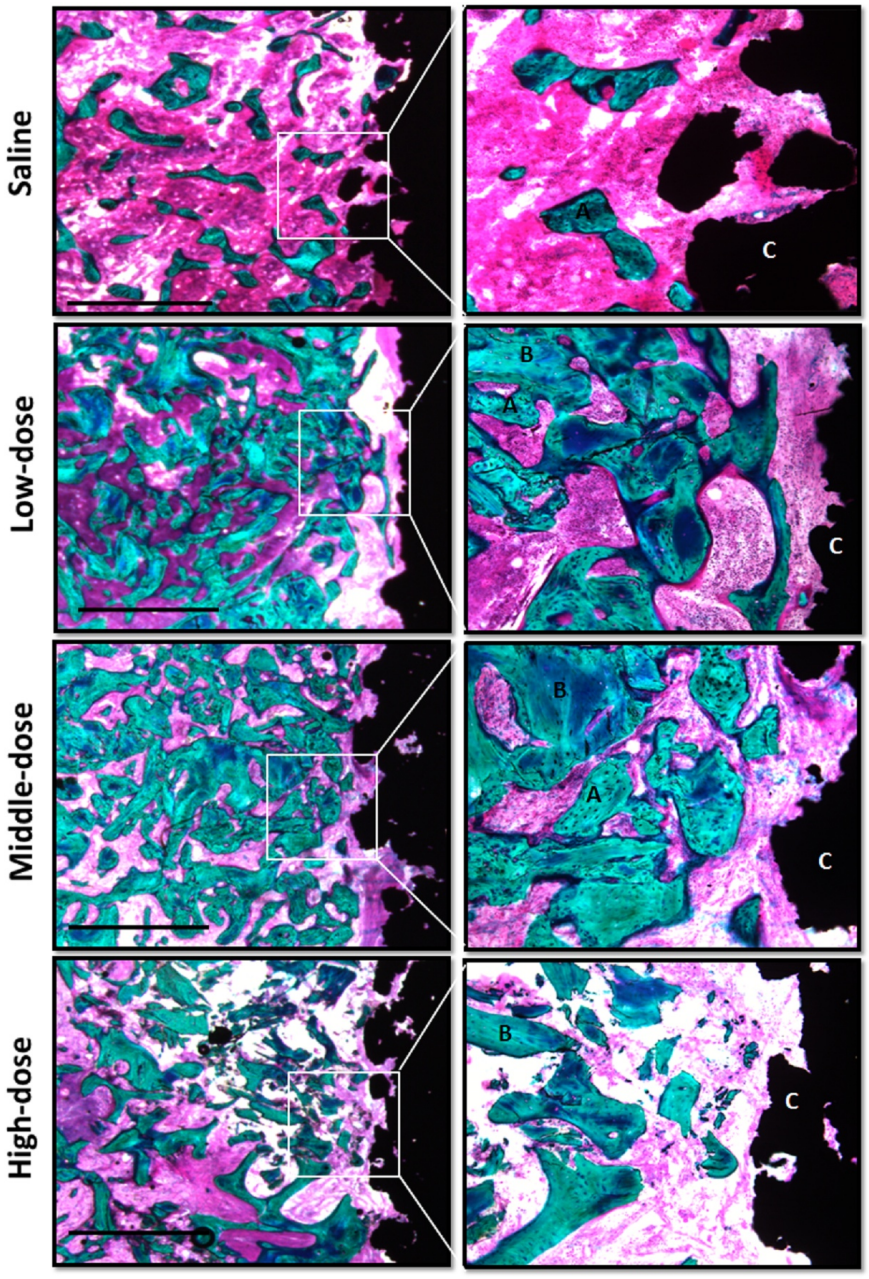


Figure 37. Representative histologic sections of allograft treatment groups are shown. The displayed sections are from four implants inserted in the same animal in Study V. Enhanced photomicrographs of the histologic sections can be seen to the right. Increased amount of bone are seen around the implants from the low- and middle-dose treatment groups. Note the allograft chips not surrounded by any new bone in the high-dose treatment group. A = New bone, B = Allograft, C = Implant. Bar = 1 mm.

amount of new bone could be due to preservation of the morselized allograft. Preservation of the allograft prolongs its osteoconductive properties and thereby facilitates new bone formation. Another explanation could be that bisphosphonate retains the newly formed bone. A third explanation could be a direct stimulatory effect of the bisphosphonate on the osteoblast. This is supported by *in vitro* studies [100,101]. It is not possible to conclude which explanation is most likely from the present studies.

β-TCP granules are an alternative to bone grafts. Little is known about the effect of combining β-TCP granules with local bisphosphonate treatment. The purpose of **Study VI** was to investigate whether soaking β-TCP granules in a zoledronate solution would increase new bone formation and implant fixation. Välimäki et al. have shown that systemic zoledronate treatment can increase new bone formation around bioactive glass microspheres implanted into the bone marrow of rats [205]. **Study VI** showed that local zoledronate treatment could increase maximum shear stiffness. However, this result was not reflected in the histomorphometrical findings. No difference in the fractions of new bone was observed between intervention and control groups. It could be that zoledronate optimized the composition of the newly formed bone and thereby increased the strength of the implant-bone interface.

Bone morphogenenic proteins (BMP) such as BMP-2 can stimulate new bone formation and increase bone allograft remodeling [136,201]. **Study X** investigated whether local zoledronate treatment could counteract the increased graft resorption without reducing new bone formation. The local zoledronate treatment in **Study X** failed to counteract the increased graft resorption induced by BMP-2. This is in contrast to other studies where local bisphosphonate and BMP treatment resulted in allograft/callus preservation and increased amount of new bone [134,135,141]. Studies using a similar canine model as used in this thesis have shown that BMP-2 has a narrow therapeutic window [137,138]. An explanation for the discrepancy between **Study X** and the literature could be the used dose of BMP-2. It could be that a lower dose of BMP-2 would have resulted in increased osseointegration. Another explanation could be the used graft density in **Study X**. Increasing the graft density will impair new bone formation [78,135].

Implant surface as vehicle for bisphosphonate delivery (Study VIII and IX)

Adjuvant therapies for augmenting implant fixation can be delivered in several ways. Systemic administration requires access from the blood circulation to the implantation site. Bleeding can influence topical soaking of the bone bed. Using the implant surface as vehicle could be a simple and reproducible way of delivery. Rodent studies have shown that zoledronate can be delivered from a poly(D,L-lactide) (PDLLA) coating [117,124,167]. The purpose of **Study VIII**

Table 13: PDLLA studies – Overview of study design

Study	*Model*	*Groups*	*Coating*	*Observation time*
VIII	Exact fit, Tibia	Control Zoledronate	Ti	12 weeks
IX	Exact fit, Tibia	Control Zoledronate	HA	12 weeks

Ti = Titanium, HA= hydroxyapatite

Table 14: Study VIII - Biomechanical results

Groups	Max shear strength, MPa	Max shear stiffness, MPa/mm	Total energy absorption, kJ/m^2
Control	3.80 (2.97;4.63)	0.95 (0,69;1,20)	18.5 (15.5;21.6)
Zoledronate	8.26 (7.11;9.40)*	1.41 (1,14;1,67)*	47.7 (38.2;57.3)*

Mean and 95%CI. *p<0.05 when compared with control

and IX was to investigate whether zoledronate delivered from a PDLLA surface coating in a canine model could increase implant fixation and osseointegration. **Study VIII** used a Ti-coated implant. The zoledronate is thought to diffuse from the coating and into the bone bed around the implant. HA-coated implants have shown superior results with respect to implant fixation and osseointegration compared to Ti-coated implants in the model used in this thesis [62]. The effects of the HA-coating occur locally at the implant-bone interface. The purpose of **Study IX** was to investigate whether it was possible to further increase fixation of HA-coated implants with the use of a zoledronate loaded PDLLA coating.

Biomechanical results

Zoledronate released from a PDLLA coating was able to increase the mechanical fixation of both Ti- and HA-coated implants (Table 14 and 15).

Histomorphometrical results

Common for **Study VIII and IX** was the preservation of lamellar bone and increased amounts of new bone around the implants. No differences were observed with respect to new bone in contact with

Table 15: Study IX - Biomechanical results

Groups	Max shear strength, MPa	Max shear stiffness, MPa/mm	Total energy absorption, kJ/m^2
Control	1.62 (1.00;2.24)	10.70 (5.22;16.14)	0.34 (0.22;0.47)
Alendronate	6.46 (4.24;8.69)*	35.70 (23.90;47.40)*	1.23 (0.54;1.92)*

Mean and 95%CI. *p<0.05 when compared with control

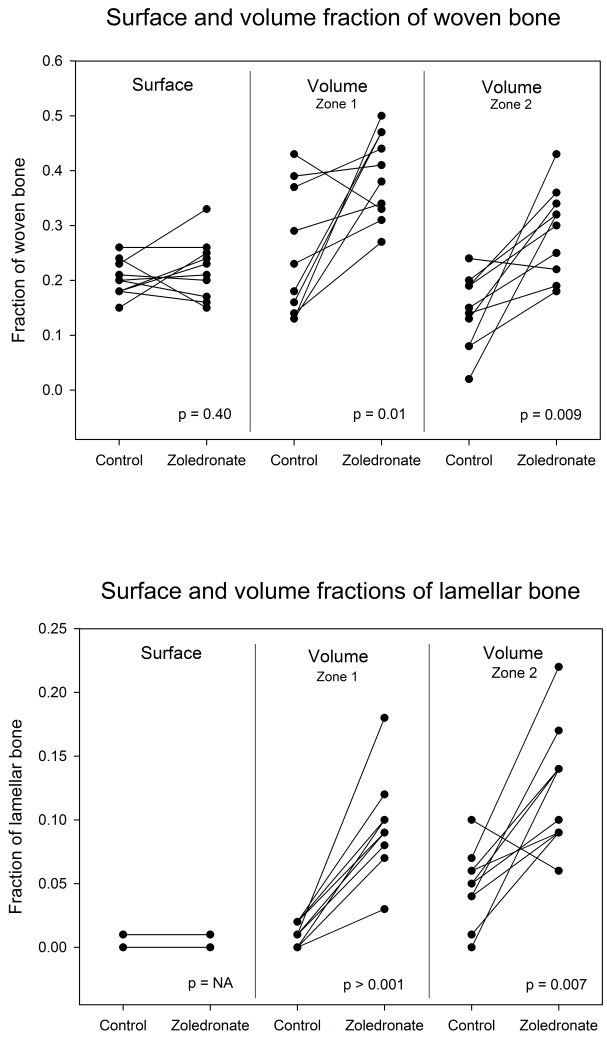


Figure 38. Study VIII. Surface and volume fraction of new and lamellar bone. Paired data connected by line. Zone 1:0-0.5 mm from surface. Zone 2: 0.5-1.0 mm from surface.

either the Ti- or HA-coated implant (Fig. 38 + 39).

Histology

A general observation was a relative dense zone of bone around the implants from the zoledronate group. This dense bone was not observed in the control group (Fig. 40).

Discussion of findings

It has previously been shown that systemic administration of alendronate can increase implant fixation in the exact-fit model used in this thesis [106]. Alendronate was administrated 2 weeks after surgery. Timing of bisphosphonate administration has been shown to be important [42]. A too early administration will have little effect. This can be explained by that lack of initial vascularization. Osteoclasts are metabolic low demanding cells and will start to resorb bone before vascularization has been fully recovered [128]. Local administration of bisphosphonate can circumvent the problem with reduced vascularization and ensure sufficient amount of bisphosphonate immediate post-operatively. Another advantage of local administration is the lack of systemic adverse effects.

Bisphosphonate can be administrated locally in two ways. The first way is to soak the bone bed in a bisphosphonate solution as done in **Study I-II and IV**. The way is simple, but is less reproducible due to bleeding from the bone bed. It is difficult to control the amount of retained bisphosphonate in the bone bed. The second way is to use the implant surface as a vehicle for delivery. This way is controlled and reproducible, but requires an implant surface coating.

PDLLA was used as surface coating in this thesis. It has previously been used as vehicle in the implant models from this thesis [159,160,206]. Furthermore, rodent studies have found it to be suitable in delivering zoledronate locally and

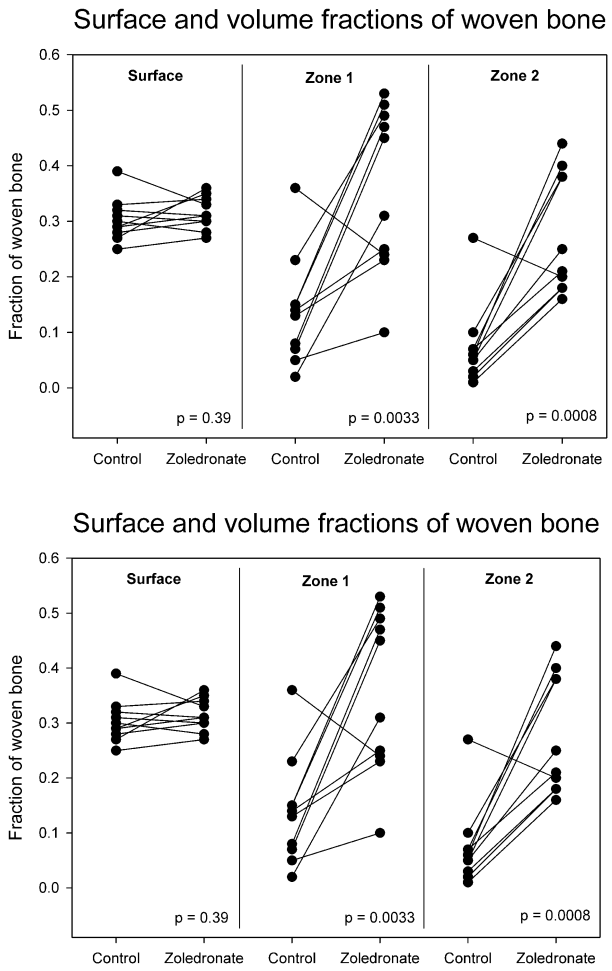


Figure 39. Study IX. Surface and volume fraction of new and lamellar bone. Paired data connected by line. Zone 1:0-0.5 mm from surface. Zone 2: 0.5-1.0 mm from surface.

increasing the amount of new bone while preserving old bone [117,124]. A drawback of using a surface coating is the lack of initial intimate contact between the "real" implant surface and bone bed.

Zoledronate increased the biomechanical implant fixation. A likely explanation for this finding is the increased amount of both lamellar and new bone around the implants. This is in correspondence with the finding of **Study II and IV** where increased biomechanical implant fixation correlated with increased peri-implant bone density.

Local delivery of zoledronate from a PDLLA coating was able to preserve lamellar bone at least 1 mm away from the implant surface. This indicates, that not all zoledronate released from the coating adheres to the bone in close contact with the implant, but some zoledronate diffuses further away form the surface.

Increased amounts of new bone around the implants were observed in **Study VIII and IX**. This is in correspondence with the finding of the other studies in this thesis and the literature[132]. It is not possible from **Study VIII and IX** to conclude whether the increased amount of new bone is due to increased osteoconductive properties of the preserved lamellar bone, a direct bone stimulatory effect of zoledronate or retaiment of newly formed bone.

A secure implant fixation is dependent on a supportive bone bed and strong implant osseointegration. **Study VIII** with a Ti-coated implant showed that zoledronate from a PDLLA coating could increase the quality of the supportive bone bed. Adding a HA-coating to a Ti-coated implant will increase implant osseointegration in the models used in this thesis [62]. **Study IX** used a HA-coated implant and investigated whether it was possible to further increase implant fixation. It was possible to increase the amount of bone around the HA-coated implants, but no effect of the zoledronate was observed at the surface. Agholme et al. have previously shown that local bisphosphonate released from a surface

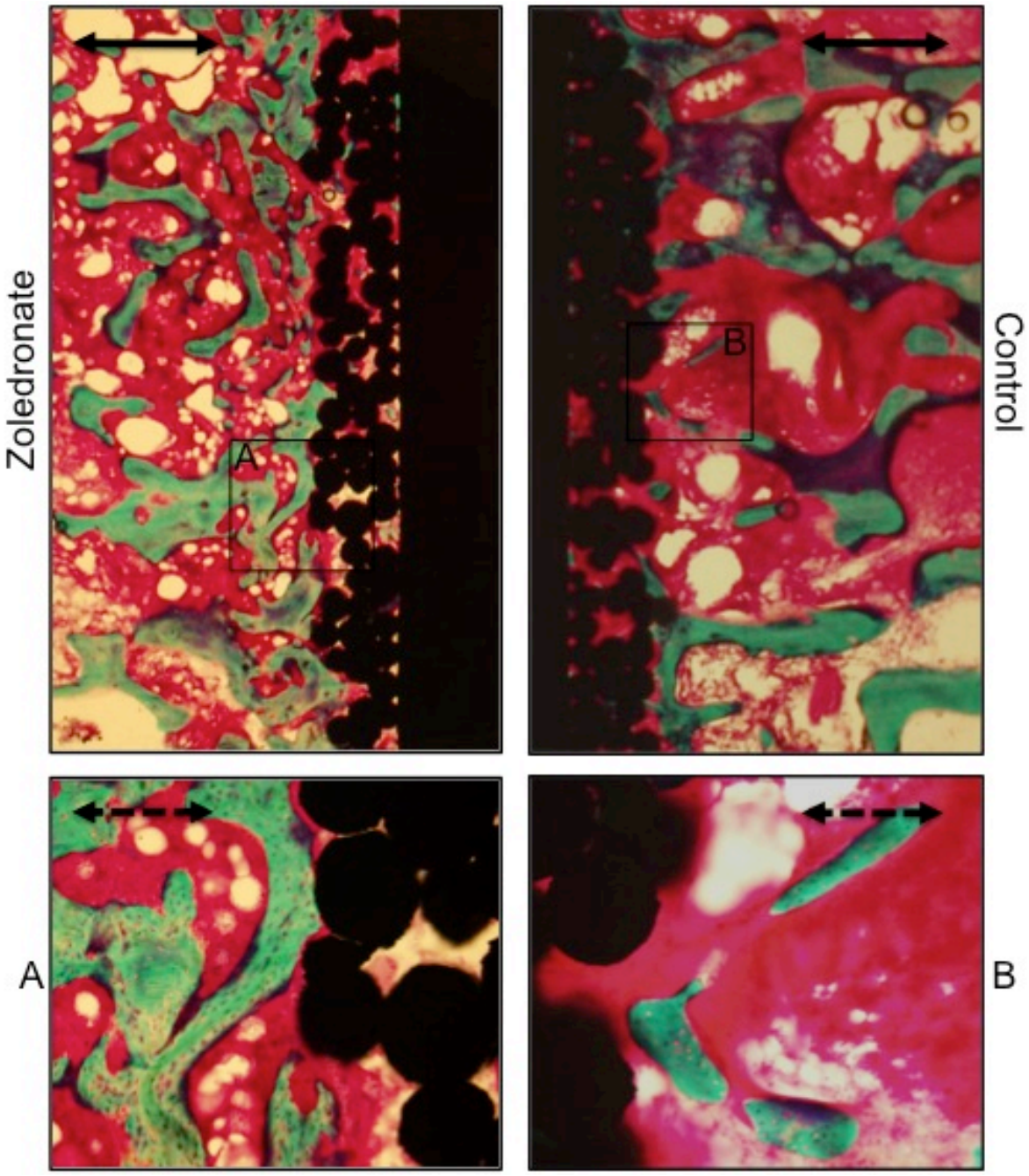


Figure 40. Representative sections from the same animal (Study VIII). Implant appears as black, marrow as red, and bone as green. Note the increased amount of bone around the zoledronate implant. No remnants of the PDLLA coating were seen. Solid bar = 1.0 mm. Dotted bar = 0.3 mm.

coating primary affects the bone bed while HA-coating primary affects implant osseointegration [123]. Furthermore, Agholme et al. found that a strong osseointegration of screws was important for torsional stability while a high bone density around the screw was important for pull-out stability. This indicates that the

Table 16: Failed osseointegration studies – Overview of study design

Study	*Model*	*Groups*	*Implant*	*Observation time*
VII	Micromotion, Femur	Post mortem time zero Micromotion	PMMA	0 weeks 12 weeks
XI	Micromotion, Femur	Control Zoledronate	PMMA	12 weeks

mechanical results obtained from **Study VIII and IX** are likely explained by the increased amount of bone around the implant and not the implant osseointegration.

Failed osseointegration and bisphosphonates (Study VII and XI)

The purpose of **Study VII** was to create a model of failed implant osseointegration. The model was intended to imitate a joint prosthesis subjected to a strain at the bone-implant interface too high to allow formation of bone and thereby prevent secondary biological implant fixation. **Study XI** investigated whether local bisphosphonate treatment could reduce the impact of implant micromotion on the surrounding bone bed.

Histomorphometrical results

Twelve weeks of micromotion resulted in resorption of lamellar bone and formation of a fibrous membrane around the implant (Fig. 41).

Local treatment with zoledronate was able to preserve some of the bone bed, but not prevent bone resorption and formation of fibrous tissue (Fig. 42).

Histology

The most striking observation was the presence of a 200-300 µm thick fibrous membrane with parallel fibers around the

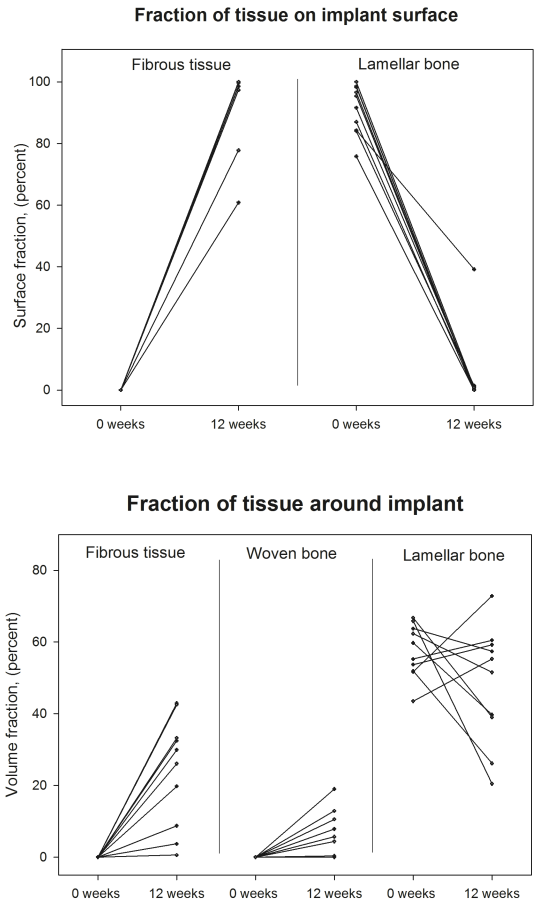


Figure 41. Study VII. Surface and volume fractions of tissue. Paired data connected by line.

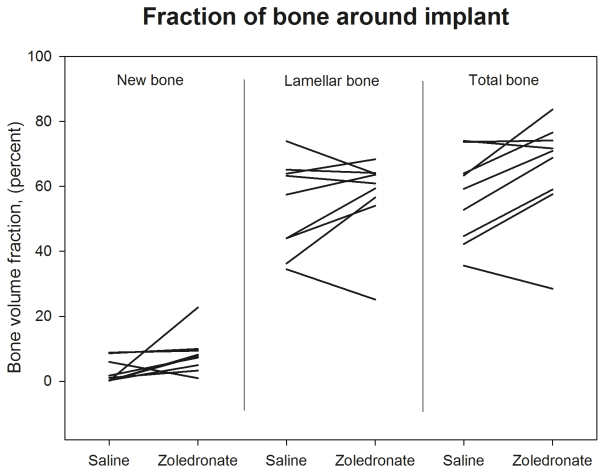


Figure 42. Study XI. Fractions of bone around implant in a 0-1 mm zone. Paired data connected by line.

micromotion implants. Local treatment with zoledronate was able to histologically reduce the thickness of the membrane, but not prevent its formation (Fig. 43).

Discussion of findings

The load induced micromotion of the implants in **Study VII** and 12 weeks of observation was able to induce resorption of bone and formation of a fibrous membrane. It has previously been shown that the amplitude of the interfacial strain dictates which tissue can be formed [47]. A too high strain will prevent formation of bone and induce formation of fibrous tissue instead. The finding of a fibrous membrane in **Study VII** was therefore expected. Micromotion without the presence of wear debris induced bone resorption around the implants. This is in correspondence with outer studies, which have shown micromotion alone is enough to induce bone resorption[53,87,207]. The model used in **Study VII** is intra-articular and with access of synovial fluid to the implant-interface. Fluid pressure alone can induce bone resorption [90,91]. It is not possible from **Study VII** to conclude whether micromotion alone or in combination with fluid flow/pressure induced bone resorption. However, **Study VII** showed that it was possible to induce bone resorption without the presence of wear-particles.

The formation of a fibrous membrane around a joint prosthesis enlarges the effective joint space. Furthermore, the membrane facilitates transportation of wear debris generation at the joint articulation to the implant-bone interface. Presence of wear debris at the interface will aggravate the osteolytic process[86,208]. There is no evidence in the literature to support that

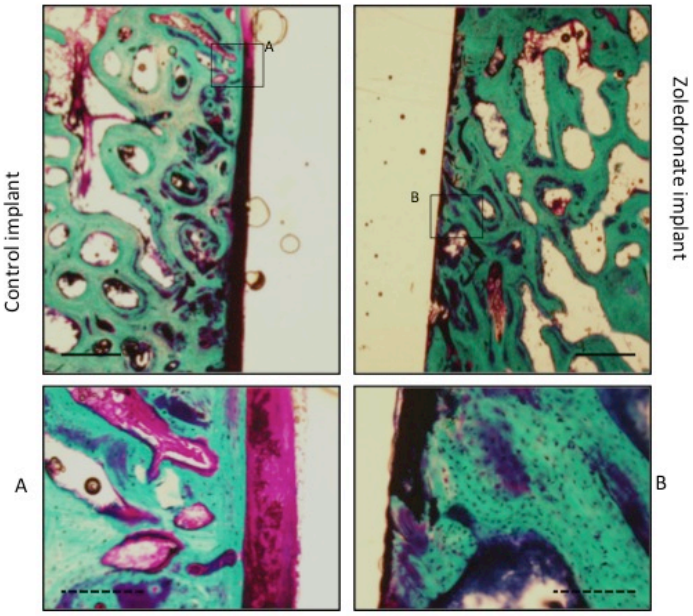


Figure 43. Representative photomicrographs of samples from the same animal (Study XI). Note the thick fibrous membrane around the control implant compared to the zoledronate implant. Solid bar = 1 mm. Dotted bar = 0.3 mm.

bisphosphonate treatment has the ability to reduce the load-induced strain and thereby allow formation of bone and implant osseointegration[111]. The rational behind bisphosphonate treatment in the context of failed osseointegration is to prevent or reduce the instability induced bone resorption and thereby prevent formation of a thick peri-prosthetic membrane, that facilitates flow of fluid and wear particles. Postponing the formation of the fibrous membrane might slow down the osteolytic process and thereby increase implant longevity.

Local bisphosphonate treatment in **Study XI** reduced the micromotion induced bone resorption, but did not prevent it. This is in agreement with rodent studies, which have shown that bisphosphonate can reduce, but not prevent, instability and fluid pressure induced bone resorption [53,142]. Several studies in this thesis have shown that bisphosphonate can preserve lamellar bone and allograft. It could be that continues micromotion is a too strong stimulus for even local zoledronate to completely prevent bone resorption. Furthermore, it could be that local zoledronate treatment only acts as a single barrier against resorption. Zoledronate, released by osteoclastic resorption, could slowly diffuse away from the bone bed. By time the protective barrier against continuously resorptive stimulus from micromotion will diminish and bone will be resorbed. Combining local bisphosphonate treatment with continuously administration might have the potential to prolong the effective barrier against bone resorption.

Conclusions on implant fixation and bisphosphonates

The specific aim of this thesis was to increase fixation and osseointegration of experimental implants. The studies included in this thesis show that local bisphosphonate treatment has the potential to increase implant fixation and osseointegration. Local bisphosphonate treatment has the ability to preserve bone and increase the amount of newly formed bone.

Bisphosphonate can preserve both autograft created *in situ* by bone compaction and morselized allograft. However, the studies in this thesis show that a dose-response relationship exists. Too high concentrations of bisphosphonate impair bone formation while optimal concentrations results in increased amounts of new bone.

Local bisphosphonate treatment can be obtained by either soaking the bone bed or using the implant surface as a vehicle. Using the implant surface as a vehicle is a controlled method to deliver bisphosphonate. It increased implant fixation and the amount of bone around the implants, but not implant osseointegration.

A model of failed implant osseointegration can be created with the use of a micromotion device, which allows an implant to piston during each gait cycle. Micromotion for 12 weeks is sufficient stimulus to induce bone resorption and formation of a fibrous membrane. Local bisphosphonate treatment can reduce

micromotion induced bone resorption, but not prevent it.

Further experimental research is needed in order to investigate the effect of different bisphosphonate doses and observation periods on implant fixation.

Future research in implant fixation and bisphosphonates

The overall aim of this thesis was to optimize implant longevity. Early implant migration is correlated with aseptic loosening [2]. Strategies to improve and accelerate secondary biological implant fixation are important. The use of bisphosphonate as an adjuvant in implant fixation is an example of a strategy that improves early implant fixation.

Correct doses of local bisphosphonate can increase implant fixation while too high doses have a detrimental effect on implant fixation and new bone formation. Few studies investigating the dose-response between bisphosphonate concentration and implant fixation have been conducted. Future dose-response studies are needed.

Soaking bone in bisphosphonate will result in some bisphosphonate to adhere the bone surface while the remaining will stay in solution between the trabeculae. Only the bond bisphosphonate will affect the osteoclast. The bisphosphonate not bond to bone has the potential to affect all cells inclusive the osteoblast. Studies investigating how different concentrations of bisphosphonate are distributed within the bone are needed.

Implant fixation is a temporal process. The uncemented implant is initially mechanical fixated. By time secondary biological osseointegration secures fixation while initial mechanical fixation is lost due to the viscoelastic properties of bone.

Manipulating biological osseointegration affects the temporal healing process. Studies including several observation periods are needed.

Load influences implant osseointegration and allograft resorption[47,80]. Most experimental implant research investigating the effects of bisphosphonate uses unloaded implant models. Load might be a strong effect modulator on bisphosphonate. It could be that loaded implant models would results in different findings than those in the literature. Studies investigating the effects of implant loading in the context of bisphosphonate treatment are needed.

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Abbreviations

ATP	Adenosin triphosphate
BMP	Bone Morphogenetic Protein
BMU	Basic multicellular unit
CV	Coefficient of Varience
DEXA	Dual-energy X-ray absorptiometry
EBRA	Ein Bild Röntgen Analyse
HA	Hydroxyapatite
PDLLA	Poly (D,L-lactide acid)
PMMA	Polymethylmethacrylate
PE	Polyethylene
ROI	Region of interest
RSA	Radiostereometric analysis
SD	Standard deviation
SURS	Systematic uniform random sampling
β-TCP	Beta-tricalcium phosphate
THA	Total hip arthroplasty
Ti	Titanium
Zol	Zoledronate

Dansk resume

I Danmark laves der årligt mere end 9000 kunstige hofter. Holdbarheden af en hofteprotese afhænger bl.a. af en sikker tidligt fiksation til knoglen. Metoder som forbedrer den tidlige fiksation af en hofteprotese har potential til at øge protesens levetid og dermed reducere risikoen for en re-operation.

Aktuelle afhandling har i experimentelle studier undersøgt om medicin, der hæmmer knoglenedbrydning, har potentiale til at øge fiksationen af en experimentel hofteprotese.

Specifikt er der blevet undersøgt om lokal behandling med bisfosfonater, som hæmmer knoglenedbrydning, kan øge fiksationen af experimentelle implatater i 4 forskellige dyremodeller:

1. *Knogle compaction* er en kirurgisk teknik, som skaber en zone af tæt pakket knogle omkring en protese. Denne afhandling har vist, at lokal behandling med bisfosfonater kan øge den mekaniske fiksation af et implant indsat med knogle compaction. Endvidere kan bisfosfonater øge mængden af knogle omkring implantatet.
2. *Knogle graft* bruges hvis der lokalt ikke er nok knogle til at kunne bære en kunstig hofte. I aktuelle afhandling har lokal behandling vist at den anvendte dosis af bisfosfonater er vigtig, når knogle graft behandles. Hvis dosis er for høj vil det hæmme knoglenydannelse. Omvendt vil en korrekt dosis kunne stimulere knoglenydannelse og øge fiksationen af en protese.
3. *Implantoverflade*n kan bruges som transportmiddel for bisfosfonater. I forskellige studier er en overflade bestående af mælkesyre blevet testet. Overfladen opløses med tiden og bisfosfonat frigives. Studierne har vist at dette kan øge mængden af knogle omkring implantaterne of dermed øge deres fiksation.
4. *Ustabile implantater* vil ikke blive fikseret i kroppen. Initial stabil mekanisk fiksation af en protese er nødvendig for at ny knogle kan vokse ind i proteseoverfladen. Knoglen omkring en ustabil protese vil med tiden blive opløst og protesen vil gå løs. I aktuelle afhandling er en model af en ustabil protese udviklet. I denne model er det undersøgt om lokal behandling med bisfosfonat kan hæmme knoglenedbrydning. Det blev fundet, at bisfosfonat kunne hæmme knoglenedbrydning, men ikke forhindre det.

Aktuelle handling danner et solidt experimentelt grundlag for at gå videre og teste lokal behandling med bisfosfonater under kliniske omstændigheder.

Resultaterne fra denne afhandling har potentielle til at øge levetiden af kunstige

hofteproteser og dermed komme
fremtidige patienter til gavn.