Aarhus University

# Research Year Report

Acid Etching and Plasma Sterilization Fail to Improve Osseointegration of Grit Blasted Titanium Implants

Mikkel Saksø Mortensen 15-01-2009

# Table of contents

Preface	3
Background	4
Implantat fixation	4
Endotoxin	4
What is endotoxin?	4
Why could endotoxin be a problem in implant surgery?	6
How can endotoxin be removed?	6
Reference List	7
Manuscript for article	8
Abstract	8
Introduction	9
Animals and Methods	9
Design	9
Animals and surgical procedure 1	10
Specimen preparation 1	10
Histomorphometrical evaluation 1	1
Mechanical testing 1	1
Statistics 1	12
Results 1	12
Surgery1	12
Histomorphometrical results 1	12
Biomechanical results1	12
Discussion 1	12
Acknowledgement1	14
Reference List	15
Figures 1	18
Tables 2	20
Legends 2	21
Perspectives 2	22
Performance report 2	23
Supervisor and co-author declaration 2	24

# Preface

This report is the result of a one-year research project carried out at Orthopedic Research Laboratory, Aarhus University Hospital from October 2007 to October 2008. The report also symbolizes the conclusion of my almost three-year long flirt with orthopedic research, starting in May 2006. In this period I have been involved in several different research projects, performed or assisted in numerous surgeries on dogs, sheep and pigs and I have found out that this is only the beginning of my research career.

I wish to thank my supervisors Kjeld Søballe, Thomas Jakobsen, Stig Storgaard Jakobsen and Jørgen Baas along with my cousin and co-researcher Henrik Saksø. Without their help this would never have been possible. And, more importantly, it wouldn't have been nearly as much fun!

Correspondence: Mikkel Saksø Mortensen Orthopedic Research Laboratory Århus University Hospital Nørrebrogade 44, Building 1A 8000 Århus C <u>Mikkel@studmed.au.dk</u>

# Background

## **Implantat fixation**

More than 70 % of all Total Hip Replacement (THA) revisions are due to aseptic loosening of the prosthesis (1).

Aseptic loosening is caused by a combination of numerous different factors. There seems to be a consensus that wear particles and micro motion of the implant play key roles, but also that endotoxin might play a greater role than earlier thought (2).

# Endotoxin

## What is endotoxin?

Endotoxin is a common name for the lipopolysaccharides (LPS) that are found in the cell walls of Gramnegative bacteria. Endotoxin is a structural part of the outer membrane of these bacteria and should not be confused with exotoxins, which are proteins that are produced and released by both gram-negative and gram-positive bacteria (3). Minute amounts of endotoxin can be released from the membrane during cell division, but being a structural component of the cell wall, significant amounts of endotoxin are only released during cell lysis (4).

As the name lipopolysaccharide indicates, endotoxin is a complex consisting of a lipid part and a saccharide component. The saccharide component consists of a long polysaccharide chain and short oligosaccharide (figure 1).



#### Figure 1

The lipid component is called Lipid A and contains the hydrophobic and membrane anchoring part of the endotoxin complex (4). Lipid A forms the external part of the lipid bi-layer in the outer membrane and is thus "shielded" from the cell's surroundings by the saccharide components (Figure 2).



#### Figure 2

Lipid A is responsible for the physiological activities of the endotoxin complex. When the cell undergoes lysis and the cell membrane disintegrates, Lipid A comes into contact with the cell's surroundings. Via Lipid A, the endotoxin complex binds to CD14 and *Toll-Like Receptor-4* (TLR-4) on the surface of macrophages. The binding triggers a signaling cascade in the macrophage, leading to the release of several potent proinflammatory cytokines. The cytokines include Interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6 and Tumor Necrosis Factor (TNF)- $\alpha$  (3).

These cytokines are interesting in relation to the bone-implant interface as they affect both osteoblasts and osteoclasts.

TNF- $\alpha$  induces differentiation of the osteoclast precursors into mature osteoclasts through a direct pathway. In osteoblasts IL-1 and IL-6 induce expression of *receptor activator of nuclear factor*  $\kappa B$  *ligand* (RANKL) and release of *macrophage colony-stimulating factor* (M-CSF). Combined, this initiates differentiation of osteoclast precursors into mature osteoclasts (3;5) (figure 3). The end result is an increase in the population of mature osteoclasts.





#### Why could endotoxin be a problem in implant surgery?

Endotoxin is known to have a strong affinity for titanium implant materials(6) and have been detected on commercially pure titanium, handled and cleaned in the same way as orthopedic titanium implants (7). Obviously, it is not desirable to insert an orthopedic implant covered with a substance known to induce osteoclast proliferation and subsequently bone resorption. Traditional sterilization using wet heat (autoclaving) lyses the cell, exposing the Lipid-A component of endotoxin to the surrounding environment, but fails to inactivate endotoxin.

#### How can endotoxin be removed?

Several methods of endotoxin removal have therefore been explored by different research groups, with plasma sterilization turning out to be efficient and possible to implement in a commercial setting (7-9).

Plasma is described as the fourth state of matter, being more energetic than the other three states; gas, liquid and solid. Because of the very high energy level in plasma a certain proportion of electrons are free rather than being bound to an atom or molecule.

Plasma sterilization does not actually include plasma, but rather a cold ionized gas. The ionized gas is less energetic than plasma and therefore contains some uncharged particles called neutrals. Among the neutrals are radicals, which in part are responsible for the sterilizing effect of the ionized gasses (10).

Plasma and ionized gas can be made by subjecting a gas to an electrical field. The electrical field induces energy to the gas which ionizes the gas atoms completely or partially. Some of the induced energy results in the release of photons and thereby both visible and ultra violet (UV) light (10).

Using oxygen  $(O_2)$  as a substrate, the ionized gas contains the reactive oxygen radicals O and  $O_2^-$ .

The effect of plasma sterilization is ascribed to three different mechanisms (10):

- 1. UV irradiation: Destructs genetic material in microorganisms.
- 2. Intrinsic photodesorption: UV photons break chemical bonds and thereby erode the molecules, atom by atom.
- 3. Etching: Reactive species, such as oxygen radicals, undergo chemical reactions with the microorganisms/molecules. This leads to the formation of volatile compounds and subsequently degradation of the microorganisms/molecules by "spontaneous etching".

# **Reference List**

- (1) Lucht U. The Danish Hip Arthroplasty Register. Acta Orthop Scand 2000 Oct;71(5):433-9.
- (2) Sundfeldt M, Carlsson LV, Johansson CB, Thomsen P, Gretzer C. Aseptic loosening, not only a question of wear: a review of different theories. Acta Orthop 2006 Apr;77(2):177-97.
- (3) Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology. Fifth ed. Philadelphia: Elsevier Mosby; 2005.
- (4) Todar K. Bacterial Endotoxin. Todar's Online Textbook of Bacteriology. Madison: 2008.
- (5) Ragab AA, Nalepka JL, Bi Y, Greenfield EM. Cytokines synergistically induce osteoclast differentiation: support by immortalized or normal calvarial cells. Am J Physiol Cell Physiol 2002 Sep;283(3):C679-C687.
- (6) Nelson SK, Knoernschild KL, Robinson FG, Schuster GS. Lipopolysaccharide affinity for titanium implant biomaterials. J Prosthet Dent 1997 Jan;77(1):76-82.
- (7) Ragab AA, Van De MR, Lavish SA, Goldberg VM, Ninomiya JT, Carlin CR, et al. Measurement and removal of adherent endotoxin from titanium particles and implant surfaces. J Orthop Res 1999 Nov;17(6):803-9.
- (8) Tessarolo F, Caola I, Nollo G, Antolini R, Guarrera GM, Caciagli P. Efficiency in endotoxin removal by a reprocessing protocol for electrophysiology catheters based on hydrogen peroxide plasma sterilization. Int J Hyg Environ Health 2006 Nov;209(6):557-65.
- (9) Lerouge S., Wertheimer M.R., Yahia L'H. Plasma Sterilization: A Review of Parameters, Mechanisms, and Limitations. Plasmas and Polymers, Vol.6, No.3, September 2001. 2001. Ref Type: Generic
- (10) Moisan M, Barbeau J, Moreau S, Pelletier J, Tabrizian M, Yahia LH. Low-temperature sterilization using gas plasmas: a review of the experiments and an analysis of the inactivation mechanisms. Int J Pharm 2001 Sep 11;226(1-2):1-21.

# **Manuscript for article**

Title

Acid etching and plasma sterilization fail to improve osseointegration of grit blasted titanium implants

Authors

Mortensen MS<sup>1</sup>, Jakobsen SS<sup>1</sup>, Sakso H<sup>1</sup>, Baas J<sup>1</sup>, Jakobsen T<sup>1</sup>, Soballe K<sup>1</sup> <sup>1</sup>Department of Orthopaedics, Aarhus University Hospital, Aarhus, Denmark

Corresponding Author: Mikkel Saksø Mortensen Orthopaedic Research Laboratory Aarhus University Hospital Nørrebrogade 44 DK – 8000 Aarhus C <u>Mikkel@studmed.au.dk</u>

Key Words: Biocompatibility, Grit blasting, Acid etching, Endotoxin, Plasma sterilization

# Abstract

Interaction between implant surface and surrounding bone influences implant fixation. We attempted to augment the bone-implant interaction by 1) adding surface micro scale topography by acid etching, and 2) removing surface-adherent pro-inflammatory agents by plasma cleaning. Implant fixation was evaluated by implant osseointegration and biomechanical fixation.

The study consisted of two paired animal sub-studies where 10 skeletally mature Labrador dogs were used. Grit blasted titanium alloy implants were inserted press fit in each proximal tibia. In the first study grit blasted implants were compared with acid etched grit blasted implants. In the second study grit blasted implants were compared with acid etched grit blasted implants that were further treated with plasma sterilization. Implant performance was evaluated by histomorphometrical investigation (tissue-to-implant contact, peri-implant tissue density) and mechanical push-out testing after four weeks observation time.

Neither acid etching nor plasma sterilization of the grit blasted implants statistically significant enhanced osseointegration or mechanical fixation in this press-fit canine implant model.

# Introduction

In the western world, more than 2% of the population over 60 years receive a total hip replacement (1) and each year one million hip replacements are inserted worldwide. Implant failure due to aseptic loosening is a serious, painful and potentially invalidating complication. More than 10% of all uncemented hip implants fail within ten years of implantation (2).

Rapid initial fixation of the implant is imperative to ensure long term implant survival (3). If the implant is not stable, micro motion between the implant and the surrounding bone will increase the risk of fibrous encapsulation of the implant (4;5), which inhibits bone ingrowth and thus increases the risk of loosening of the implant.

The macro-scale porosity of the implant surface plays an important role in osseointegration of the implant (6). Furthermore, in recent years the concept of using micro-scale topography to stimulate bone forming osteoblasts has been investigated by several different groups (7-9). Applying micro-scale topographical changes onto clinically rough surfaces has; however, proven difficult.

Etching of titanium implants makes it possible to apply micro-topographical changes onto macro-scale porosities (10). In vivo studies has shown greater osseointegration of acid etched implants compared to non-etched control implants (11-13). Furthermore, etching a grit blasted surface eliminates the inherent problem with residue of the blasting ceramic particles left on the implant, by which the particle load in the peri-implant space is reduced.

In addition, superior implant biocompatibility will further improve osseointegration. Endotoxins are molecules from the cell walls of bacteria, that induce a massive inflammatory response from macrophages leading to fibrous capsule formation (14). Implants surrounded by a fibrous capsule have an increased probability of loosening, compared to prostheses that are directly anchored in bone (15). Endotoxins have previously been found extensively on the surface of commercially available implants (16). Consequently, increasing the biocompatibility and reducing the probability of implant loosening by removal or inactivation of the endotoxins from the surface of the implants (14) by plasma sterilization appears very promising (17;18).

We hypothesized that acid etching would improve early osseointegration and implant fixation of grit blasted implants, as evidenced by improved biomechanical implant fixation, increased formation of new bone, and decreased presence of fibrous tissue. Furthermore, we hypothesized that removing the bioburden by plasma sterilization of the implants would improve these parameters additionally.

# **Animals and Methods**

## Design

## (Figure 1)

The study consisted of two separate sub-studies, both designed as paired animal studies. Ten skeletally mature Labrador dogs were used. Mean body weight was 34 kg [range 25 kg: 39 kg]. Each dog had two

implants inserted in each proximal tibia. In the upper tibia site, we investigated the effect of surface etching on grit blasted implants. In the lower tibia site we investigated the effect of combining surface etching and plasma sterilization on grit blasted implants. Due to denser bone quality, the upper tibia surgical site offered a better biomechanical implant fixation compared to the lower site (p=0.0098). Hence, no cross comparisons between the two sub-studies was performed. The observation time was four weeks.

## Animals and surgical procedure

Under general anaesthesia, using sterile technique, the proximal part of tibia was exposed through an anteromedial extraarticular approach. Two Kirschner (K) wires were inserted perpendicular to the surface with 15.0 mm in between. The most proximal K wire was inserted with the implant centre 15 mm from the tibia plateau. The K-wires guided the 5.5 mm cannulated drill creating 11.0-mm-deep holes. Drilling was performed at two rotations per second to prevent thermal trauma to the bone. All bone debris and soft tissue was removed from the drill hole before the implant was inserted. Finally, the soft tissue was closed in layers. Pre-and postoperatively, the dogs were given one dose of Cefuroxim, 1.5 g intravenously, as antibiotic prophylaxis. A fentanyl transdermal patch (75  $\mu$ g/h) lasting three days was given as postoperative analgesic treatment. The dogs were allowed full weight bearing postoperatively. The dogs were bred for scientific purposes, and the study was approved and monitored by the Danish Animal Research Committee.

# The implants

#### (Figure 2)

The aluminum oxide  $(Al_2O_3)$  grit blasted implants (L = 10 mm, Ø = 6 mm) were made out of Ti-6Al-4V alloy (Depuy Inc., Warsaw, IN, USA), and were prepared as follows.

Acid etching: Acid etching was done at room temperature in an acidified NaF solution for six minutes. The implants were then rinsed with a 1% Alconox (Alconox, Inc., White Plains, NY, USA) and 2% Liquinox (Alconox, Inc., White Plains, NY, USA) detergent at 45°C for 30 minutes. The implants were then rinsed three times in 15 minute cycles with RO-H<sub>2</sub>O at 45°C. Finally the implants were dried at 60°C.

Plasma sterilization: The plasma sterilization with pure  $O_2$  gas was performed in a 500 Watt plasma chamber (7200 RF Plasma Processing System; PVA TePla America, Inc.) with a gas flow rate of 250 standard cubic centimetres per minute (sccm) and a chamber pressure of 300 mTorr (40 x  $10^{-5}$  bar). The cycle time was 30 minutes.

After the preparation, the implants were passivated, packaged separately and sterilized using standard DePuy manufacturing methods.

## **Specimen preparation**

After four weeks observation the animals were sacrificed with an overdose of hyper saturated barbiturate and the proximal part of tibia was excised and cleaned and thereafter stored at  $-20^{\circ}$ C. The outermost 0.5 mm of the implant-bone specimen was cut off and discarded. The rest of the implant with surrounding bone was divided into two sections perpendicular to the long axis of the implant with a water-cooled diamond band saw (Exact Apparatebau, Nordenstedt, Germany). The outermost section was cut to a thickness of 3.5 mm and stored at  $-20^{\circ}$ C pending mechanical testing (19). The innermost part 6.0 mm was prepared for histomorphometry. The specimens were dehydrated in graded ethanol (70-100%) containing basic fucsin, and embedded in methylmetacrylate (Technovit 7200 VCL; Exact Apparatbau, Nordenstedt, Germany). Four vertical, uniform, random sections were cut with a hard-tissue microtome (KDG-95; MeProTech, Heerhugowaard, The Netherlands) around the centerpart of each implant as described by Overgaard et al. (20). Before making the sections, each implant was rotated randomly around its axis. The sections were cut parallel to this axis. The 40-µm-thick sections were counterstained with 2% light green (BDH Laboratory Supplies, Poole, UK) and then mounted on glass (21). This preparation provides red staining of non-calcified tissue and green staining of calcified tissues such as woven bone and lamellar.

## **Histomorphometrical evaluation**

#### (figure 3)

Blinded histomorphometric analysis was done using a stereological software program (C.A.S.T.-grid Olympus Denmark A/S, Ballerup, Denmark). Fields of vision from a light microscope were captured on a computer monitor and a user-specified grid was superimposed on the microscopic fields. Four vertical sections representative of each implant were analyzed and cumulated (20).

With the aid of the software, we defined two zones in the sections. Zone 1 stretches from the implant surface and 500  $\mu$ m outwards into the surrounding tissue, and Zone 2 was defined starting 500  $\mu$ m from the same implant outer surface line and extending another 500  $\mu$ m outwards into the surrounding tissue.

In both zone 1 and 2 tissue volume fractions were quantified by point counting (22). On the implant surface, line intercept-technique with sine-weighted lines was used to estimate the bone-implant contact (23).

Intra-observer variation was determined as coefficients of variation on double measurements on four randomly selected implants. All the coefficients were well within the expected range; however, defining small amounts of new bone on the implant surface and discriminating between new and lamellar bone close to the implant proved troublesome (new bone in zone 1 [24%], lamellar bone on the surface [52%]).

## **Mechanical testing**

The thawed implants were tested to failure by axial push-out test on a MTS Bionics Test Machine (MTS, Eden Prairie, MN, USA). The specimens were placed on a metal support jig with a 7.4 mm diameter central opening. The implant was centralized over the opening, assuring a 0.7 mm distance between the implant and the support jig. The direction of loading was from the cortical surface inward. A preload of 2 N was applied to standardize contact conditions before initiating loading. We used a displacement rate of 5 mm/min with a 2,5 kN load cell. Load and deformation were registered by a personal computer. Each specimen length and diameter was measured with a micrometer screw gauge and used to normalize pushout parameters (24). Ultimate shear strength (MPa) was determined from the maximal force applied until failure of the bone-implant interface. Apparent stiffness (MPa/mm) was obtained from the slope of the linear section of the curve. Energy absorption (kJ/m<sup>2</sup>) was calculated from the area beneath the curve until failure.

# **Statistics**

Histomorphometrical as well as mechanical data followed a normal distribution and a paired t-test was used to distinguish between groups. The data were presented as means with standard deviation (SD). Differences between means were considered statistically significant for p-values less than 0.05.

## **Results**

## **Surgery**

All dogs were fully weight bearing within three days of surgery. No postoperative complications were seen, and no dogs were excluded during the observation period. No signs of infection were seen during the harvesting of the specimens.

## **Histomorphometrical results**

(Table 1 and 2)

There were no statistically significant differences between Grit Blasted and Grit Blasted+Acid Etched (substudy 1), or Grit Blasted and Grit Blasted+Acid Etched+Plasma Sterilized (sub-study 2) in regards to any of the tissues compared in any of the zones measured.

All implants were well osseointegrated, with almost 50% of the implant surface in direct bone contact in sub-study 1 (upper site) and almost 40% implant to bone contact in sub-study 2 (lower site). Furthermore, there was no fibrous tissue in the bone-implant interface in sub-study 1, and only 5% in sub-study 2.

## **Biomechanical results**

#### (Table 3)

By gross observation, all implants were well anchored in the bone. No statistically significant differences were found between neither Grit Blasted and Grit Blasted+Acid Etched nor Grit Blasted+Acid Etched+Plasma Sterilized in any parameter.

# Discussion

The purpose of this study was to improve implant performance by acid etching micro-textural changes on grit blasted implant surfaces together with the effect of removing surface-adherent pro-inflammatory agents by plasma sterilization.

In this study, we showed that neither etching alone, nor etching combined with plasma sterilization improved the osseointegration and the fixation of the grit blasted titanium implants in this press-fit canine implant model.

The canine implant model was chosen because canine cancellous bone closely resembles human cancellous

bone (25). The paired design of the two sub-studies eliminates the influence of interindividual differences between the test animals. All implants were placed extra-articularly and not exposed to direct loading, which limits this model in its representation of a clinical joint replacement. The model is; however, well established and has in numerous publications shown a good ability to detect subtle differences in mechanical fixation and osseointegration caused by surface modifications (26-28). Grit-blasted implants was chosen because they represent a well-recognized, clinically applied surface for uncemented joint replacement components (29;30).

Previous studies have shown positive effect of acid etching titanium implants, using a similar canine implant model (26). However, both the baseline treatment of the implants and the etching technology were different. Daugaard et al. used a dual etching technique on a chemically milled surface, whereas we used single etching on a commercial available grit blasted surface. Better performing control coatings together with a slightly increased under-drilling (0.1 mm – 0.5 mm) in our setting may explain the increased bone-to-implant contact in our study (more than 50% in both groups in sub-study 1) compared to Daugaard et al. (control: 18.4%, etched: 36.2%). This excellent degree of osseointegration may have been difficult to further improve.

As previously reported, we also observed that etching reduced the amount of grit blasting debris on the implant surface (31). We did not see any short-term benefits of this in terms of osseointegration and early implant fixation.

Although clearing the implant surface of endotoxin theoretically would improve osseointegration, we found no positive effect of plasma sterilizing the etched implants. This may very well reflect the fact that there is a dose dependent response to endotoxin. Endotoxin tends to accumulate on polyethylene, and the negative effect of polyethylene wear particles can partly be ascribed to surface-adherent endotoxin (32).

Furthermore, after insertion of an endotoxin free implant, endogenous endotoxins will adhere to its surface. If an implant contaminated with exogenous endotoxin is inserted, some of the endotoxin will be cleared from the implant. This potentially establishes a comparable steady state concentration of endotoxin on the surface of both intervention and control implants (33).

Even though acid etching did not improve the initial osseointegration in this study, the reduction of alumina particles on the implant surface may prove beneficial long term by reducing the amount of particulate metal in the peri-prosthetic tissue (34). In clinical practice, only a small portion (10% to 20%) of the uncemented press-fit implant surface is in bone contact (35). Therefore improvement of the remaining 80% - 90% should be investigated in a model where 'over-drilling' introduces a small gap between the bone and the implant surface. Furthermore, to test the effect of the bio burden, it would be relevant to perform a dose-response study.

Neither acid etching nor plasma sterilization of the grit blasted implants statistically significant enhanced osseointegration or mechanical fixation. Two theoretically attractive ways of improving implants performance failed to prove efficient in this press-fit canine implant model. All implants performed

extremely well both in regards of osseointegration and mechanical fixation presenting a likely explanation for the lack of any proven benefits by acid etching and plasma sterilization.

# Acknowledgement

The authors wish to thank Depuy Orthopaedics Inc. for financial and material support. The authors would also like to thank laboratory technicians Jane Pauli and Annette Milton for excellent laboratory work.

## **Reference List**

- (1) Lucht U. The Danish Hip Arthroplasty Register. Acta Orthop Scand 2000 Oct;71(5):433-9.
- (2) Malchau H, Herberts P, Eisler T, Garellick G, Soderman P. The Swedish Total Hip Replacement Register. J Bone Joint Surg Am 2002;84-A Suppl 2:2-20.
- (3) Karrholm J, Borssen B, Lowenhielm G, Snorrason F. Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. J Bone Joint Surg Br 1994 Nov;76(6):912-7.
- (4) Aspenberg P, Herbertsson P. Periprosthetic bone resorption. Particles versus movement. J Bone Joint Surg Br 1996 Jul;78(4):641-6.
- (5) Soballe K, Hansen ES, Rasmussen H, Jorgensen PH, Bunger C. Tissue ingrowth into titanium and hydroxyapatite-coated implants during stable and unstable mechanical conditions. J Orthop Res 1992 Mar;10(2):285-99.
- (6) Bobyn JD, Pilliar RM, Cameron HU, Weatherly GC. The optimum pore size for the fixation of poroussurfaced metal implants by the ingrowth of bone. Clin Orthop Relat Res 1980 Jul;(150):263-70.
- (7) Att W, Tsukimura N, Suzuki T, Ogawa T. Effect of supramicron roughness characteristics produced by 1- and 2-step acid etching on the osseointegration capability of titanium. Int J Oral Maxillofac Implants 2007 Sep;22(5):719-28.
- (8) Owen GR, Jackson J, Chehroudi B, Burt H, Brunette DM. A PLGA membrane controlling cell behaviour for promoting tissue regeneration. Biomaterials 2005 Dec;26(35):7447-56.
- (9) Zinger O, Zhao G, Schwartz Z, Simpson J, Wieland M, Landolt D, et al. Differential regulation of osteoblasts by substrate microstructural features. Biomaterials 2005 May;26(14):1837-47.
- (10) Fandridis J, Papadopoulos T. Surface Characterization of Three Titanium Dental Implants. Implant Dent 2008;17(1):91-9.
- (11) Cho SA, Park KT. The removal torque of titanium screw inserted in rabbit tibia treated by dual acid etching. Biomaterials 2003 Sep;24(20):3611-7.
- (12) Hacking SA, Harvey EJ, Tanzer M, Krygier JJ, Bobyn JD. Acid-etched microtexture for enhancement of bone growth into porous-coated implants. J Bone Joint Surg Br 2003 Nov;85(8):1182-9.
- (13) Klokkevold PR, Johnson P, Dadgostari S, Caputo A, Davies JE, Nishimura RD. Early endosseous integration enhanced by dual acid etching of titanium: a torque removal study in the rabbit. Clin Oral Implants Res 2001 Aug;12(4):350-7.
- (14) Greenfield EM, Bi Y, Ragab AA, Goldberg VM, Nalepka JL, Seabold JM. Does endotoxin contribute to aseptic loosening of orthopedic implants? J Biomed Mater Res B Appl Biomater 2005 Jan 15;72(1):179-85.

- (15) Santavirta S, Xu JW, Hietanen J, Ceponis A, Sorsa T, Kontio R, et al. Activation of periprosthetic connective tissue in aseptic loosening of total hip replacements. Clin Orthop Relat Res 1998 Jul;(352):16-24.
- (16) Ragab AA, Van De MR, Lavish SA, Goldberg VM, Ninomiya JT, Carlin CR, et al. Measurement and removal of adherent endotoxin from titanium particles and implant surfaces. J Orthop Res 1999 Nov;17(6):803-9.
- (17) Tessarolo F, Caola I, Nollo G, Antolini R, Guarrera GM, Caciagli P. Efficiency in endotoxin removal by a reprocessing protocol for electrophysiology catheters based on hydrogen peroxide plasma sterilization. Int J Hyg Environ Health 2006 Nov;209(6):557-65.
- (18) Lerouge S., Wertheimer M.R., Yahia L'H. Plasma Sterilization: A Review of Parameters, Mechanisms, and Limitations. Plasmas and Polymers, Vol.6, No.3, September 2001. 2001.
  Ref Type: Generic
- (19) Linde F, Sorensen HC. The effect of different storage methods on the mechanical properties of trabecular bone. J Biomech 1993 Oct;26(10):1249-52.
- (20) Overgaard S, Soballe K, Jorgen H, Gundersen G. Efficiency of systematic sampling in histomorphometric bone research illustrated by hydroxyapatite-coated implants: optimizing the stereological vertical-section design. J Orthop Res 2000 Mar;18(2):313-21.
- (21) Gotfredsen K, Budtz-Jorgensen E, Jensen LN. A method for preparing and staining histological sections containing titanium implants for light microscopy. Stain Technol 1989 May;64(3):121-7.
- (22) Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988 May;96(5):379-94.
- (23) Baddeley AJ, Gundersen HJ, Cruz-Orive LM. Estimation of surface area from vertical sections. J Microsc 1986 Jun;142(Pt 3):259-76.
- (24) Soballe K. Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs. Acta Orthop Scand Suppl 1993;255:1-58.
- (25) Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 1998 Feb;139(2):663-70.
- (26) Daugaard H, Elmengaard B, Bechtold JE, Soballe K. Bone growth enhancement in vivo on press-fit titanium alloy implants with acid etched microtexture. J Biomed Mater Res A 2008 Jan 9.
- (27) Elmengaard B, Bechtold JE, Soballe K. In vivo study of the effect of RGD treatment on bone ongrowth on press-fit titanium alloy implants. Biomaterials 2005 Jun;26(17):3521-6.
- (28) Zainali K, Danscher G, Jakobsen T, Jakobsen SS, Baas J, Moller P, et al. Effects of gold coating on experimental implant fixation. J Biomed Mater Res A 2008 Mar 11.
- (29) Delaunay C, Kapandji AI. Survival analysis of cementless grit-blasted titanium total hip arthroplasties. J Bone Joint Surg Br 2001 Apr;83(3):408-13.

- (30) Goldberg VM, Stevenson S, Feighan J, Davy D. Biology of grit-blasted titanium alloy implants. Clin Orthop Relat Res 1995 Oct;(319):122-9.
- (31) Szmukler-Moncler S, Testori T, Bernard JP. Etched implants: a comparative surface analysis of four implant systems. J Biomed Mater Res B Appl Biomater 2004 Apr 15;69(1):46-57.
- (32) Cho DR, Shanbhag AS, Hong CY, Baran GR, Goldring SR. The role of adsorbed endotoxin in particleinduced stimulation of cytokine release. J Orthop Res 2002 Jul;20(4):704-13.
- (33) Tatro JM, Taki N, Islam AS, Goldberg VM, Rimnac CM, Doerschuk CM, et al. The balance between endotoxin accumulation and clearance during particle-induced osteolysis in murine calvaria. J Orthop Res 2006 Nov 14.
- (34) Bohler M, Kanz F, Schwarz B, Steffan I, Walter A, Plenk H, Jr., et al. Adverse tissue reactions to wear particles from Co-alloy articulations, increased by alumina-blasting particle contamination from cementless Ti-based total hip implants. A report of seven revisions with early failure. J Bone Joint Surg Br 2002 Jan;84(1):128-36.
- (35) Geesink RG. Osteoconductive coatings for total joint arthroplasty. Clin Orthop Relat Res 2002 Feb;(395):53-65.

# **Figures**

Figure 1



#### Figure 2



## Figure 3



# **Tables**

Table 1:

Sub-study 1	New Bone		Lamellar Bone	
Surface	Mean	(SD)	Mean	(SD)
GB	48.8	(8.4)	6.0	(4.2)
GB+AE	47.4	(11.3)	8.2	(8.3)
P-values	0.786		0.443	
Zone 1	Mean	(SD)	Mean	(SD)
GB	28.9	(5.0)	16.7	(7.2)
GB+AE	26.9	(7.9)	17.3	(9.0)
P-values	0.540		0.868	
Zone 2	Mean	(SD)	Mean	(SD)
GB	18.2	(6.2)	21.7	(3.8)
GB+AE	19.0	(7.9)	21.6	(10.0)
P-values	0.826		0.972	

#### Table 2:

Sub-study 2	New Bone		Lamellar Bone	
Surface	Mean	(SD)	Mean	(SD)
GB	35.4	(6.7)	11.2	(5.8)
GB+AE+PS	39.6	(6.1)	9.3	(3.8)
P-values	0.502		0.473	
Zone 1	Mean	(SD)	Mean	(SD)
GB	18.3	(2.1)	16.4	(6.4)
GB+AE+PS	17.5	(1.9)	13.8	(4.1)
P-values	0.796		0.281	
Zone 2	Mean	(SD)	Mean	(SD)
GB	12.7	(4.4)	18.4	(6.2)
GB+AE+PS	14.7	(6.5)	16.7	(3.6)
P-values	0.468		0.530	

Table 3:

	STRENGTH (MPa)		ENERGY (kJ/m²)		STIFFNESS (MPa/mm)	
Sub-study 1	Mean	(SD)	Mean	(SD)	Mean	(SD)
GB	10.15	(1.61)	1.05	(0.46)	73.12	(21.14)
GB+AE	9.41	(0.90)	1.18	(0.52)	65.60	(13.82)
P-values	0.148		0.572		0.380	
Sub-study 2	Mean	(SD)	Mean	(SD)	Mean	(SD)
GB	7.02	(3.21)	0.61	(0.47)	64.57	(24.41)
GB+AE+PS	6.95	(1.77)	0.61	(0.31)	63.67	(16.23)
P-values	0.944	1	0.969	)	0.925	5

# Legends

Figure 1: Placement of the implant pairs in the right and left tibia.

Figure 2:

SEM pictures of the implant surfaces:

In picture d, crystals of aluminum oxide ( $Al_2O_3$  particles) from the grit blasting are clearly visible. Pictures e and f show the pits created by the etching process and the pictures indicate that the majority of the particles has been removed by the etching process.

Figure 3:

Picture showing a histological cross section used for Histomorphometry. The close-up picture shows Lamellar Bone (L) and New Bone (N) in contact with the implant surface. Marrow is marked (M).

Table 1: Sub-study 1 (upper site). Tissue area and volume fractions. Surface (0  $\mu$ m): Tissue area fraction in percent. Zone 1 (0-500  $\mu$ m): Tissue volume fraction in percent. Zone 2 (500-1000  $\mu$ m): Tissue volume fraction in percent.

Table 2: Sub-study 2 (lower site). Tissue area and volume fractions. Surface (0  $\mu$ m): Tissue area fraction in percent. Zone 1 (0-500  $\mu$ m): Tissue volume fraction in percent. Zone 2 (500-1000  $\mu$ m): Tissue volume fraction in percent.

Table 3: Data of mechanical parameters.

# Perspectives

We did not find any effect of removing endotoxin from the implants in this study, but this in no way means that endotoxin can be taken off the list of possible causes for aseptic loosening.

First of all, as stated in the background-section, endotoxin has never been thought to be the sole cause for aseptic loosening, but more likely a contributing factor along with wear particles, micro motion and several other factors.

Secondly, the study was very short (only four weeks) compared to the several years it usually takes for aseptic loosening to develop in human patients.

Finally, the study was performed in dogs, which however much their bone structure resembles human bone, will never be completely representative of human patients.

To further investigate the effect of endotoxin in relation to aseptic loosening some changes in the study setup seems relevant.

Our research group has previously used implant models that incorporate both wear particles and micro motion. Using such a model would be a natural next step in the research process.

A future study should incorporate a dose-response investigation with a different amount of endotoxin on each implant group. This could bring us closer to understanding the levels of endotoxin that are required to provoke a response in the bone-implant interface. When inserting implants covered with known amounts of endotoxin, it would be very interesting to measure the endotoxin amount on the surface of the implants after extraction. This would shed some light on the "steady state" theory. If the implants that were endotoxin-free when inserted have as much endotoxin on the surface as the implants loaded with endotoxin, it would indicate that there is no reason to clear the implants of endotoxin, and that future research should focus on some of the other factors involved in aseptic loosening.

# **Performance report**

The research student was trained to such extend that he either independently or under supervision are able to perform all aspects of the study.

This includes:

- Planning and outlining a scientific project
- Writing a protocol
- Preparation of surgery
- Surgical techniques
- Specimen preparation
- Histomorphometry
- Biomechanical testing
- Fundraising
- Data analysis
- Writing of report and scientific article

The student attended following courses:

- 2006 iNANOschool Nanobiocompatibility 5-day intensive PhD course, Aarhus University, Denmark
- 2007 Introduction to research year, Aarhus University, Denmark
- 2007 Literature searching, Aarhus University, Denmark
- 2007 Basic Laboratory Animal Science, Aarhus University, Denmark
- 2007 Bone Biology, Aarhus University, Denmark
- 2008 Basic biostatistics; brush-up, Aarhus University, Denmark

The student attended following meetings:

- 2007 Danish Orthopaedic Society, Spring Meeting, Aarhus, Denmark
- 2008 Orthopaedic Research Society (ORS), 54th Annual Meeting, San Francisco, USA
- 2008 # Danish Orthopaedic Society, Spring Meeting, Aalborg, Denmark
- 2008 \* Nordic Orthopaedic Federation (NOF), 54th Congress, Amsterdam, The Netherlands
- 2008 \* International Society of Orthopaedic Surgery and Traumatology (SICOT), 24th Triennial World Congress, Hong Kong, China
- 2008 # Medical Students Research Society (SMS), Congress, Sandbjerg, Denmark
- 2008 Danish Orthopaedic Society, Autumn Meeting, Copenhagen, Denmark
- 2009 \* Orthopaedic Research Society (ORS), 55th Annual Meeting, Las Vegas, USA (abstract accepted)

# Oral Presentation \* Poster Presentation

The student achieved following awards and nominations as a co-author:

- 2008 Poster Presentation Price, SICOT, 24th Triennial World Congress, Hong Kong, China
- 2008 Poster Award Nomination, NOF, 54th Congress, Amsterdam, The Netherlands

# Supervisor and co-author declaration

Kjeld Søballe Professor, MD, DMSc Main supervisor Jørgen Baas MD, Ph.d. Supervisor

Thomas Jakobsen MD, Ph.d. Supervisor Stig Storgaard Jakobsen MD, Ph.d. Supervisor Henrik Saksø Medical/Research student Co-author