No positive effect of Acid Etching or Plasma Cleaning on osseointegration of titanium implants – a ten canine study

Research Year Report

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Preface

This research year project is based on a scientific study performed at The Orthopaedic Research Laboratory, Aarhus University Hospital. My scientific work began with a project involving impurities, e.g. endotoxins, on the surface of orthopedic implants (see protocol below). Unfortunately new amendments in the American law made it administratively more complicated for the medical industry (Zimmer, Inc.) to support medical scientific work. This led half way through the research year to a project change as a necessity to finish a study within the given timeframe. The new project was very similar to the initial one but in addition to removing impurities it mainly involved a surface modification that modifies the topography of the implant surface.

The outcome of my research year in addition to this report and useful research experience is a manuscript for an article which will be submitted to the Journal *International Orthopaedics*. Additionally this project has taken me around the world for presentations at various congresses and beside that has given me a valuable clinically insight into orthopedic surgery by independently operating and assisting operations for other projects.

I would like to thank my main supervisor, Professor Kjeld Søballe, for giving me an invaluable opportunity to learn about and to be a part of science and scientific work. A big thanks to my project supervisors, co-authors, lab technicians and everyone else at the laboratory for making it a pleasant, memorable and beneficial year – socially as well as professionally.

DePuy, Warsaw, IN, USA kindly donated the implants and funded the work for my final study. Additional financial support was gratefully received from The Helga and Peter Korning Foundation and DOS Foundation. A scholarship was received from The Ministry of Science, Technology and Innovation.

Background

Worldwide the need of arthroplastic surgery is considerable. Around one million hip replacements are made each year and in Denmark the total number of primary total hip alloplastics (THA) in 2006 was 70.000 (1). More than 2 % of the population over 60 years in Denmark has a THA (18). In older people, about 5 % of the implants will fail within 10 years, while the failure percentage for younger, physically active people in the same time span is as high as 20 (14). Implant failure is treated with replacement of the components in a revision arthroplasty procedure. The survival of the revision implants is even worse than of the primary arthroplasties (21). Throughout the last 30 years there has been an increasing interest in the development of uncemented orthopedic implants and in Denmark 51 % of all primary THA in 2006 were uncemented (1). One of the challenges when implanting uncemented THA is the initial fixation, as there is compelling evidence emphasizing that early fixation is essential to long term survival of an implant (17). The earlier fixation and thus less inflammatory response and less micro motion of implant the lower risk of formation of a fibrous capsule around the implant and thus lower risk of implant loosening and failure (23).

Implant surface modification may affect implant fixation and by improving implant biocompatibility reduce the risk of long term implant failure. Topographical changes to the implant surface may affect cellular adhesion and proliferation. Acid etching is a surface modification technique that modifies the surface topography on the micrometer scale leading to a greater roughness with potential to enhance implant osseointegration (11).

Plasma cleaning the implant surface removes impurities such as endotoxins which may affect early fixation. Recent research has shown that endotoxins can be an overseen problem, when orthopedic implants are inserted in humans (12). Endotoxins are small amphipatic molecules from the cell wall of bacteria and the classic endotoxin is Lipopolysaccharide (LPS) from Gram negative bacteria. Endotoxins are powerful stimulators of immune responses as they induce macrophages and other cells to release interleukins and several other factors to create a massive inflammatory reaction. In contrast to the bacteria, the endotoxins remain intact and active even after conventional sterilizing procedures.

Research in the improvement of THA is relevant and beneficial. Particularly for the younger patients but improvements will also benefit a relatively large, and more importantly, steadily growing group of elderly patients. The socioeconomically benefit can be substantial, as it might reduce the number of revisions and as a further aspect; THA is a reconstructive procedure, enabling the patient to return their profession and be economically self-supportive.

Endotoxin project

The endotoxin project had my focus in the first half of my research year. Below selected parts from the endotoxin protocol:

Does endotoxin affect osseointegration of orthopedic implants?

The aim of the study is to investigate the role of endotoxins adherent to the implant surface and test the following hypothesis: Endotoxin free implants will have improved early fixation compared to conventionally sterilized prostheses defined experimentally by 1) improved mechanical fixation, 2) increased formation of new bone and, 3) reduced occurrence of fibrous tissue.

Study A: Performed by research student Mikkel Saksø Mortensen.
The aim of study A is to investigate the effect of endotoxin in a simple setting. This is done in an unloaded implant model consisting of four implants. The implants are surface treated with different concentrations of LPS-endotoxin with the aim of showing a possible dose response relationship between endotoxin and impaired implant fixation.

Study B: Performed by research student Henrik Saksø.
Study B is closer to a clinical setup, due to the load bearing characteristics as the implants are placed intra-articularly and therefore subjected to load, stress and joint fluid pressure along with a greater inflammatory reaction (synovitis). This model may polarize the potential difference in the dose response model in study A and furthermore show the effect of load and motion on osseointegration of the LPS-endotoxin free implants.

Design
The study is designed as a randomized, paired, controlled animal experiment with 10 dogs. We use dogs, as the architecture and composition of canine bone is very similar and comparable to human bone (2;9).

Study A
Two implants will be inserted in each humerus, which gives four implants per dog. The four implants are surface treated in different ways:
Implant 1: Sterilized and defatted and cleared of endotoxin (Ragab's method).
Implant 2: Sterilized and defatted and therefore expected to have endotoxin on the surface.
Implant 3: Sterilized and defatted and coated with LPS-endotoxin.
Implant 4: Sterilized and defatted and coated with 2 x concentration of LPS-endotoxin.

Study B

In each medial femural condyle one implant will be inserted, which gives two implants per dog. The two implants are surface treated in different ways:

Implant 1: Sterilized and defatted and cleared of endotoxin (Ragab's method).

Implant 2: Sterilized and defatted and therefore expected to have endotoxin on the surface.

After an observation period of 4 weeks, the dogs are terminated and the bones containing the implants will be harvested and frozen. Every implant will be examined mechanically by a push-out-test and microscopically by histomorphometry.

Materials and methods

Dogs:

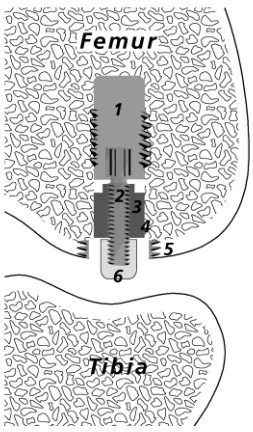
Ten skeletally mature "American Hound"-canines (20-25kg).

Implants:

Study A

40 cylinder-shaped (6 x 10 mm) plasma-spray coated titanium implants (Ti-6A1-4V) with a 0,5 mm gap. The implants are divided into four groups of ten, which are surface treated as described above.

Study B



20 sets of a loaded implant system including an anchor screw (no.1+2) on which a cylinder-shaped (6 x 10 mm) plasma-spray coated titanium test implant (Ti-6A1-4V) with a 0,75 mm gap is attached (no. 3). A centralizing ring (no. 5) is inserted in the cortical bone and a polyethylene (PE) plug (no. 6) is placed on top of the ancor screw so that the test implant is loaded. The gap between the PE plug and centralizing ring will allow synovial fluid to access the bone-to-implant interface (no. 4). The implants are divided into two groups of ten, which are surface treated as described above.

After the operation, the dogs are allowed unrestricted movement with full load on the operated limbs. The dogs receive analgetic treatment until pain free. The wounds are attended daily.
Four weeks after the surgery, the dogs are terminated with an overdose of hyper saturated pentobarbital. The bones containing the implants are removed from the dogs and kept at -20 degrees Celsius until specimen preparation.

Preparation

Using a Struers Accutom™ the implants with surrounding bone are cut in two pieces:
- The proximal 3,5 mm part is refrozen for use in the push-out test.
- The distal 6,5 mm is stored in 70 % alcohol at 5 degrees Celsius for use in histomorphometry.

Work schedule

The project will be based at *Orthopaedic Research Laboratory, Aarhus University Hospital, Aarhus.* Here sawing of implant-bone blocks, preparation of implants (casting and dying) and mechanical and histological testing will take place. The implant-group of this laboratory, led by Professor Kjeld Søballe, has substantial experience in use of the model described above and has previously carried out internationally recognized research on similar animal models (26).
The canine model has previously been used with great success to investigate the effect of bone growth stimulating hormones and new implant coatings.

The breed of experimental animals, preoperative preparation of implants, surgical procedures, postoperative observation, termination and extraction of implant-bone blocks will take place at the *Orthopaedic Biomechanics Laboratory, Minneapolis, MN, USA* in cooperation with Professor Joan Bechtold.
The treating of implant surfaces, including clearing of endotoxins, will take place at *Edward Greenfield's laboratory at Case Western Reserve University, University Hospitals of Cleveland, Cleveland, OH 44106-5000, USA.*

Time schedule

Preparation of endotoxin-free/LPS-coated implants (USA)	Fall 2007
Surgery (USA)	Fall 2007
Sawing of bone/implant blocks (DK)	Winter 2007
Mechanical testing (DK)	Winter 2007
Histomorphometry preparation (DK)	Winter 2007
Histomorphometry (DK)	Winter 2007
Data analysis (DK)	Spring 2008
Publication	Summer 2008

Participants

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Medical student/Research student Henrik Saksø

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Manuscript for article[1]

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[1] For the purpose of easy reading a few deviations from *Instructions for Authors* (*International Orthopaedics*) is made.

Abstract

Objective

Implant surface treatments that improve early osseointegration may prove useful in long-term survival of uncemented implants. We investigated Acid Etching and Plasma Cleaning on titanium implants.

Materials and Methods

In a randomized, paired animal study, four porous coated Ti implants were inserted into the femurs of each of ten dogs.

1. PC (Porous Coating; control)
2. PC+ET (Acid Etch)
3. PC+ET+PLCN (Plasma Cleaning)
4. PC+PSHA (Plasma Sprayed Hydroxyapatite; positive control)

After four weeks mechanical fixation was evaluated by push-out test and osseointegration by histomorphometry.

Results

The PSHA-coated implants were better osseointegrated than the three other groups ($p<0.05$). Within the deep implant porosity, there was more newly formed bone in the control group compared to the ET and ET+PCLN groups ($p<0.05$).

Conclusions

In terms of osseointegration PC+PSHA was superior to the other three groups. Neither the acid etching nor the plasma cleaning offered any advantage in terms of implant osseointegration.

Introduction

Worldwide the need of arthroplastic surgery is considerable. Around one million hip replacements are made each year and in Denmark the number of primary hip replacements is increasing (18). There are studies indicating that early fixation is essential to long term survival of an implant (17;22). Surface modification is a factor that may affect implant fixation, and investigation of new and better implant surface modifications is beneficial for improving implant biocompatibility and thereby reducing the risk of implant failure.

One way to improve fixation could be by acid etching the implant surface. Acid etching modifies the surface topography on the micrometer scale leading to a greater roughness with potential to enhance implant osseointegration (11). This treatment has been investigated in several studies, both *in vitro* and *in vivo* and the general findings were that acid etching creates a surface that enhance cell proliferation and differentiation (19;27), as well as giving a relatively higher bone-to-implant contact, a better bone ingrowth and better osseointegration of experimental orthopedic titanium implants (5;6;13).

Another way to improve fixation could be by plasma cleaning the implant surface to remove impurities such as endotoxins. This bio burden can be an overseen problem, when orthopedic implants are inserted in humans (12). Significant amounts of endotoxins have been found on the surface of implant components sterilized by the manufacturer (20). Endotoxins are small amphiphatic molecules from the cell wall of bacteria and the classic endotoxin is Lipopolysaccharide (LPS) from Gram negative bacteria. Endotoxins are aggressive inflammatory agents that may contribute to implant failure by aseptic loosening (4). New methods of clearing endotoxins from the surface of prostheses have recently been developed (20) and in this study these impurities are tried removed with a specific plasma cleaning procedure.

The purpose of this canine study was to evaluate the effect of a specific acid etch surface treatment and plasma clean surface treatment on experimental titanium implants. We hypothesize that these surface modifications would improve biomechanical implant fixation and osseointegration.

Materials and Methods

Design

The study was a randomized, paired and controlled animal experiment with 10 dogs. Two implants were inserted into the distal part of each femur. One in each medial and one in each lateral epicondyle (fig. 1). Bone quality was assumed by equal between all four implant locations. The four implants were surface treated in different ways: Porous coating as a control, porous coating with Plasma Sprayed Hydroxyapatite as a positive control, porous coating with Acid Etching Surface Treatment and porous coating with Acid

Etching and Plasma Cleaning Surface Treatment. After an observation period of 4 weeks, the dogs were terminated and the bones containing the implants were harvested and frozen. Every implant was examined mechanically by push-out-test and microscopically by histomorphometry.
Prior to performing the study approval was obtained from our National Animal Research Inspectorate.

Animals

Ten skeletally mature Labrador canines, with a mean body weight on 34 kg [range 25 kg: 39 kg] and an age of 14-15 months. All canines were female and specifically bred for research purpose.

Implants

Custom made cylindrical Titanium alloy core implants (Ti-6A1-4V, Ø = 6 mm, L = 10 mm) with commercially pure titanium porous coating and four different surface treatments were used. Coating and surface treatments were all applied by DePuy Inc., Warsaw, IN, USA (table 1). All Ti6Al4V substrates are per ASTM F-136. All Titanium beads are per ASTM F-67. All beads are attached by a sintering process with heat and pressure handling in vacuum furnace. Hydroxyapatite was deposited by Plasma Arc Process.

Acid Etch Surface Treatment

The implants were treated at room temperature for 6 minutes in an acidified NaF solution to form micro-scale texturing on the bead surface. Then the implants were soaked and rinsed with a detergent containing 1 % Alconox and 2 % Liquinox (Alconox Inc., White Plains, NY, USA) at 45^{o} C for 30 minutes and then for three consecutive 15 minute treats with RO-H_2O at 45^{o} C. The implants were dried at 60^{o} C.
Evaluating the surface of the Acid Etched implants was done by applying the technology on a polished surface. Average surface roughness was 0.15 μm and XPS (X-ray Photoelectron Spectroscopy) confirmed that none of the chemicals were incorporated in the surface oxide layer.

Plasma Cleaning Surface Treatment

Implants were passivated prior to the cleaning process. Plasma cleaning was done in a plasma chamber (7200 RF Plasma System; PVA TePla America, Inc.) under following conditions: A cycle time of 30 mins, a O_2-gas flow rate at 250 sccm, a chamber pressure of 300 mTorr, and a power of 500 Watts.

Plasma Sprayed Hydroxyapatite

Implants were passivated prior to the coating process. Plasma spraying with Hydroxyapatite (HA) created implants with following specifications: A thickness of 40-60 μm, a tensile strength of 81.0 MPa, a HA crystallinity of 78.4 wt %, a Ca/P ratio of 1.67, a HA wt. % of 96.71, and a TCP wt. % of 3.29.

Surgical procedure

Under general anesthesia using sevoflurane and using general sterile conditions, the femoral epicondyles were exposed starting with a 3 cm medial incision. A 2.0 mm guide wire was placed perpendicular to the epicondylar surface, 15 mm from the distal edge of the condyle and 10 mm from the anterior edge of the condyle. A cannulated drill bit of 5.5 mm diameter was then used to create an 11 mm deep drill hole. A drill speed of 2 rotations per second was used to avoid thermal trauma to the bone. Same procedure was repeated for the lateral epicondyle. In each drill hole, the implants were inserted press fit by light hammer blows with a specially designed implant inserter tool to secure uniform axial placement. Finally, the soft tissues were closed in layers and 10 ml Bupivacaine was given as local infiltration analgesic. The procedure was repeated for the opposite side. Pre- and postoperatively, the dogs were given one dose of Cefuroxim, 1.5 g intravenously as antibiotic prophylaxis. A Fentanyl transdermal patch (75 µg/h) lasting three days was given as postoperative analgesic treatment. All animals were postoperatively allowed unlimited activity and unrestricted movement. The wounds were attended daily. After a four week observation period, the dogs were sedated and euthanized with an overdose of hypersaturated barbiturate. The bones containing the implants were removed from the dogs and kept at -20 degrees Celsius until specimen preparation.

Preparation

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 (Exakt Apparatebau; Norderstedt, Germany). The outermost 3.5 mm is refrozen for use in the mechanical test. The innermost 6.0 mm is stored in 70 % alcohol at 5 degrees Celsius for use in histological analysis. The specimens were dehydrated in graded ethanol (70-100 %) containing 0.4 % basic fuchsine (Merck, Darmstadt, Germany), and embedded in methyl methacrylate (MMA; Merck, Hohenbrunn, Germany). From the MMA block four vertical, uniform, random sections were cut with a hard-tissue microtome (Leiden, KDG-95; MeProTech, Heerhugowaard, The Netherlands) around the centre part of each implant. Before making the sections, the MMA block was rotated randomly around its axis to avoid biased estimates. The 50-µm-thick sections were counterstained with 2 % light green (BDH Laboratory Supplies, Poole, UK) and then mounted on glass. This preparation provides red staining of non-calcified tissue and green staining of calcified tissues.

Mechanical testing

Implants were tested to failure on an axial push-out test machine (858 Mini Bionix; MTS, Eden Praire, 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 thereby assuring a 0.7 mm distance between the implant and the support jig (8). The direction of loading was from the cortical surface inward. The implant was pushed

through the opening by a 5.0 mm diameter probe with a displacement rate of 5 mm/min on a 10 kN axial load cell. Each specimen length and diameter was measured with a micrometer and used to normalize push-out 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 (J/m^2) was calculated from the area beneath the curve until failure. All push-out parameters were normalized by the cylindrical surface area of the transverse implant section tested.

Histomorphometrical analysis

Blinded histomorphometrical analysis was done using a stereological software program (CAST 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. The specimen preparation procedure and the grid system provided highly reliable results with negligible bias (3). We defined two regions of interest: Zone 0 from implant core surface and 250 µm outwards, and zone 1 from a line between the core implant and "the outermost bead" and 750 µm outwards. In zone 0 and zone 1 bone-implant contact was defined as the implant surface covered with bone and estimated using sine-weighted lines. In zone 1 bone volume was estimated by point counting. Intraobserver variation was determined as coefficients of variation on double measurements on randomly selected implants (table 3).

Statistics

Intercooled STATA 8.0 software (StataCorp., College Station, TX) was used. All data followed a normal distribution and fulfilled the assumptions for one-way ANOVA. Data analyzed with ANOVA (statistically significant difference between any of the groups within same parameter) was followed by Students paired t-test. Differences between means were considered statistically significant for p-values less than 0.05.

Results

Surgery

No postoperatively complications were seen and all canines were fully weight bearing within 3 days of surgery. All animals completed the four week observation period. At the implant sites there were no clinical signs of infection.

Biomechanics

None of the four groups was statistical significantly different from others within any of the parameters (table 2).

Histology

All implants seemed osseointegrated and lamellar bone was evenly distributed around the drill cavity (fig. 2). More new bone was apparent in the deep implant porosity in the HA coated implants, while more fibrous tissue was apparent in the deep implant porosity in the acid etched implants. In general only sparse amount of fibrous tissue was seen outside the porous coating. No sign of delamination of the HA coating.

Histomorphometry

Statistically significant more new bone was observed on the surface in zone 0 of PC and PC+PSHA implants compared to the PC+ET and PC+ET+PLCN implants (fig.3). No difference in the amount of new bone on the surface in zone 0 was observed between PC and PC+PSHA or between PC+ET and PC+ET+PLCN.
Statistically significant more new bone was observed on the surface in zone 1 of PC+PSHA compared to the other three coatings (fig.4). No statistically significant differences in volume fractions were found in zone 1.

Discussion

The aim of this study was to investigate a specific Acid Etch Surface Treatment and Plasma Cleaning Surface Treatment on porous coated titanium implants in a well established canine model of osseointegration (10;24). We found no advantage of ET and ET+PLCN compared to the control group but we found that PSHA-coated implants were better osseointegrated than the other three groups.

The experimental model used represents the part of cementless joint replacements placed in cancellous bone. We used a non-weight-bearing setup which is relevant at this point of investigation. Compared to a weight-bearing setup it lacks the more clinically relevant conditions as direct load and joint fluid pressure, but still it is a well-controlled and easier to standardize setup. Although load is not directly applied, the implants are susceptible to load through the biomechanical energy transmission of the bone. Canines were used, as the architecture and composition of canine bone is more similar and comparable to human bone than of many other animals (2;9). A four week observation period in this model has previously shown to be sufficient to reveal statistically significant difference (16;25). Four implants each with one of the four different surface treatments were inserted in each dog, making each dog its own control and thereby preventing interspecies variation. By using PSHA-coated implants as positive control we validate our model used by again establishing a positive effect (24).

No statistically significant difference was found in the biomechanical data within any of the parameters. This could be to the fact that the implants were inserted press fit and all implants initially were in close contact with bone making a potential difference difficult to be detected within the observation period. This could be an explanation why we found no correlation between biomechanical and histomorphometrical data as we have previously found using this model (15).

Within the deep implant porosity, we found more newly formed bone in the control groups compared to the ET and ET+PCLN groups. This difference may be due to a toxic effect of not rinsed away acidic remnants of the ET or simply that this specific ET creates a less biocompatible surface. The ET process was evaluated with XPS by applying the treatment on a polished surface, meaning that the surfaces of the ET implants were not directly evaluated.

Using this model other investigators have previously shown a positive effect of ET (7) and now we find no or poorer effect of ET. The technology used in the two different experiments was not similar procedures with regards to implant surface texture, acidic solutions, cycle-times, temperatures, rinsing and drying, so it is not directly comparable. This diversity in outcome calls for further studies on this level of investigation and shows the importance of optimizing the technology before setting up clinical trials.

No effect of PLCN was observed on ET implants. The purpose of PLCN was to reduce the amount of impurities e.g. endotoxins. Another way to reduce the amount of endotoxins on an implant surface could be with acidic passivation (20;28). It is difficult with the present data to conclude whether the lack of effect is due to comparison of two already "endotoxin reduced" surfaces due to ET or no effect of PLCN itself. This taking into account an interesting perspective would be to have an intervention group with PC+PLCN to be able to compare it to both the control PC group and the PC+ET group.
Another interesting perspective would be to insert the implants in a gap model, making it potentially easier to detect a small difference in new bone formation.

In conclusion, this study indicates that the acid etched implants in this model have a reduced biocompatibility compared to non-acid etched implants.
Furthermore, this study shows that HA coating is superior to non-HA coated implants with respect to biocompatibility.
The lack of positive effect of acid etching compared to previous findings in our group emphasizes the need of preclinical research before using an experimental outcome in a clinical trial.
Conclusion on the effect of PLCN on non-acid etched implants should be done with caution, since the non-acid etched implant surface could contain higher amounts of impurities.

Acknowledgements

We thank Anette Milton and Anna Bay Nielsen from Department of Orthopaedics, Aarhus University Hospital for their skilful technical assistance. DePuy, Warsaw, IN, USA donated the implants and funded the work.

Figures and Legends

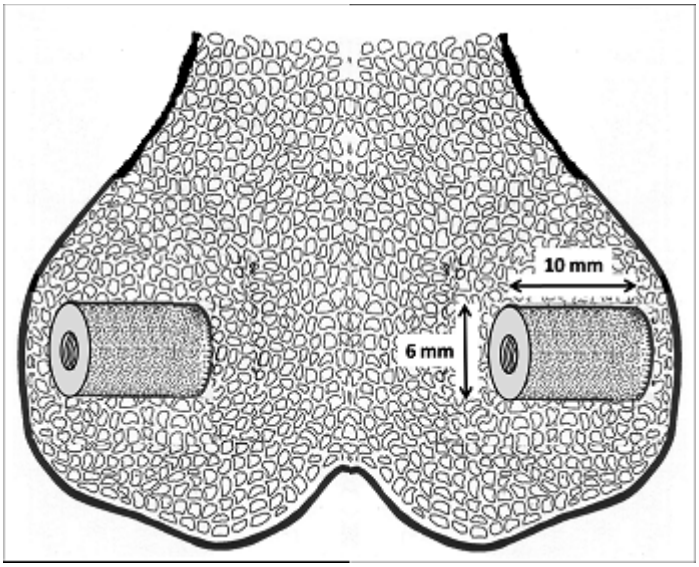

Fig. 1. Schematic drawing of implants inserted press fit into femoral epicondyles.

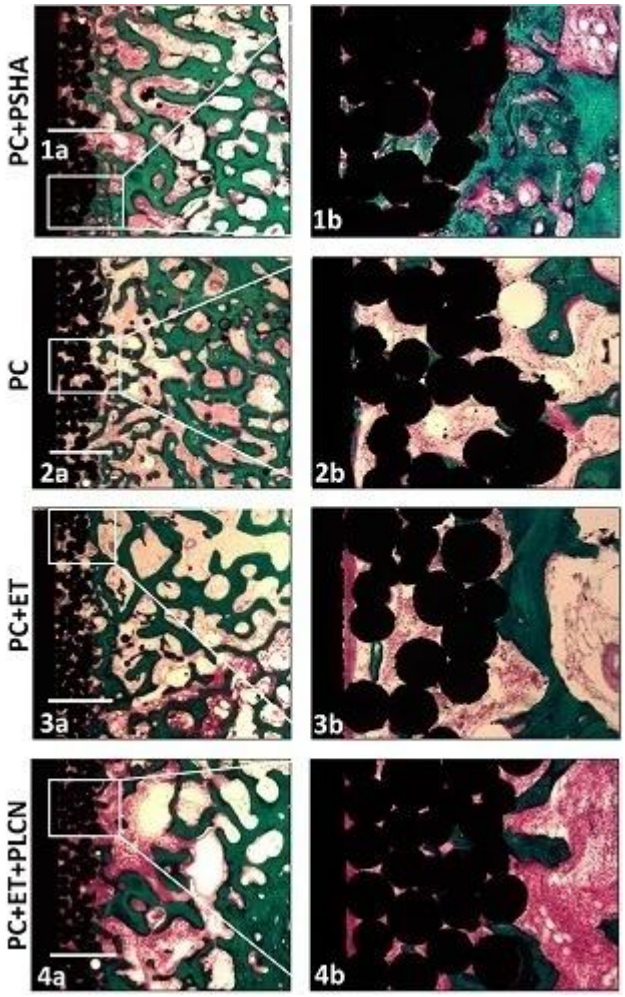

Fig. 2. Representative histological sections of the four different treatment groups.
More new bone was seen on the PSHA implants in both regions of interest (1a+b) compared to the intervention groups (3a+b and 4a+b). The control goup (2a+b) had more new bone in the deep implant porosity compared to the intervention groups (3a+b and 4a+b). Both of the etched implant groups showed a distinct rim of fibrous tissue along the core implant (3a+b and 4a+b).
Bar = 1 mm.

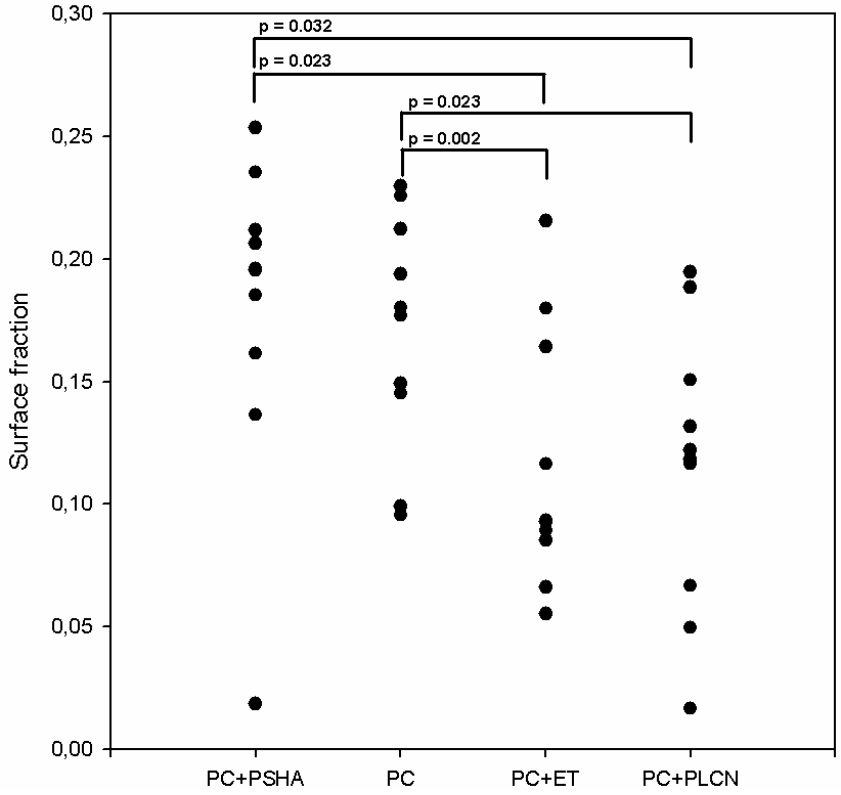

Fig. 3. Histomorphometry; new bone surface fraction in zone 0.

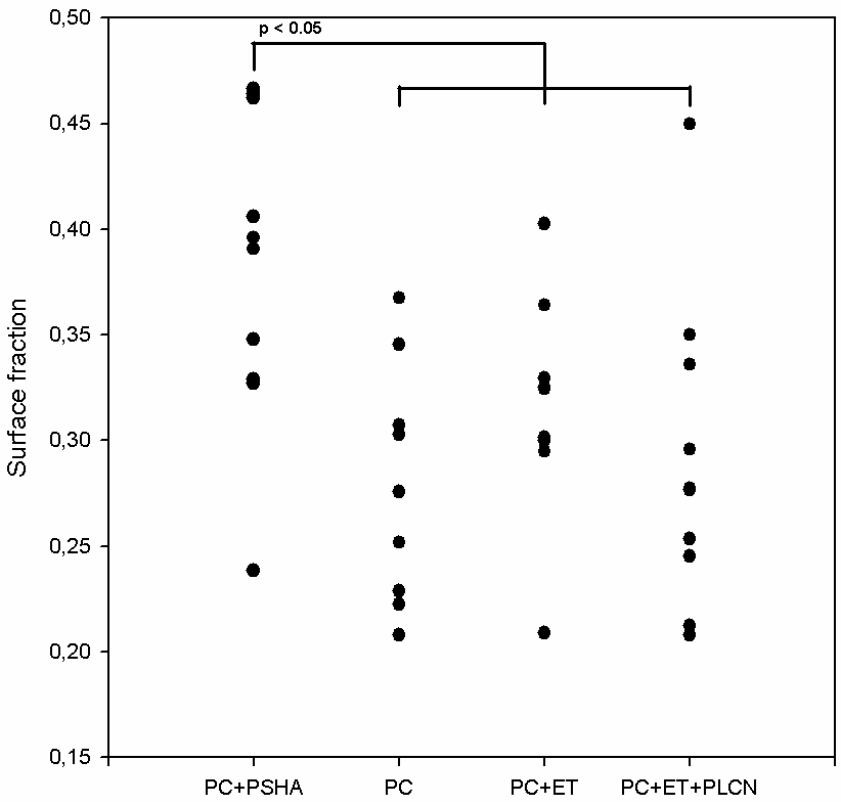

Fig. 4. Histomorphometry; new bone surface fraction in zone 1.

Table 1. Implant specifications

Implant type	Coating Material - 1	Coating Material - 2	Post processing	Porosity / Avg. Pore size
Porous Coating	Spherical Ti beads	None	None	40-50% / 250~300 µm
Porous Coating + PSHA	Spherical Ti beads	Plasma Sprayed Hydroxyapatite	None	40-50% / 250~300 µm
Porous Coating + ET	Spherical Ti beads	None	Acid Etch surface treatment	40-50% / 250~300 µm
Porous Coating + ET + PLCN	Spherical Ti beads	None	Acid Etch surface treatment + Plasma Cleaning surface treatment	40-50% / 250~300 µm

Table 2. Biomechanical push-out data (parametric), *mean (SD)*

Implant	Ultimate shear strength (MPa)*		Apparent stiffness (MPa/mm)**		Total energy absorption (MJ/m^2)***	
PC	21.72	*(4.70)*	109.73	*(41.50)*	4.23	*(1.14)*
PC+PSHA	24.07	*(4.03)*	129.37	*(25.96)*	4.69	*(1.07)*
PC+ET	22.71	*(6.56)*	113.25	*(52.21)*	4.85	*(1.64)*
PC+ET+PLCN	22.00	*(6.92)*	118.71	*(41.54)*	4.15	*(1.58)*

*p = 0.742 (ANOVA)
**p = 0.734 (ANOVA)
***p = 0.430 (ANOVA)

Table 3. Variation Coefficient (CV %)

	Fibrous Tissue	New Bone	Lamellar Bone	Marrow Space
Zone 0 (area)	< 1 %	< 1 %	-	< 1 %
Zone 1 (area)	-	< 1 %	41 %	< 1 %
Zone 1 (vol.)	-	< 1 %	< 1 %	< 1 %

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Perspectives and future studies

Even though the results from the etching study are opposite our hypothesis, it is still very useful. The negative findings emphasize the importance of pre clinical and experimental research. Before implementing any experimental findings in clinical trials thorough investigation would be advisable through pre clinical research.

Considering the results from the etching study a straightforward perspective would be adding an intervention group to the setup where a porous coated titanium implant is plasma cleaned without the preceding acid etching (PC+PLCN). By this we could be able to investigate the effect of plasma cleaning solely (PC compared to PC+PLCN) and also to extend the investigation when combining plasma cleaning with acid etching (PC+PLCN compared to both PC+ET and PC+ET+PLCN).

Regarding the design of the etching study a perspective is modifying the implant system from press fit insertion to gap insertion. This would reduce the influence of existing bone (old bone) in the fixation and osseointegration of the implant simplifying the histomorphometry.

Another straightforward perspective when taking the course of my research year into account is to complete the etching study. However further scientific research awaits my graduation.

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:

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:

2008 **Poster Presentation Price**, SICOT, 24th Triennial World Congress, Hong Kong, China

2008 **Poster Award Nomination**, NOF, 54th Congress, Amsterdam, The Netherlands

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