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Bone growth enhancement *in vivo* on press-fit titanium alloy implants with acid etched microtexture

Henrik Daugaard¹, Brian Elmengaard¹, Joan E. Bechtold², and Kjeld Soballe¹

¹Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Aarhus University Hospital, Norrebrogade 44, Building 1A, DK-8000 Aarhus C, Denmark

²Orthopaedic Biomechanics Laboratory, Midwest Orthopaedic Research Foundation and Minneapolis Medical Research Foundation at Hennepin County Medical Center, 914 South Eighth Street, Minneapolis, Minnesota 55404

Abstract

Early bone ongrowth secures long-term fixation of primary implants inserted without cement. Implant surfaces roughened with a texture on the micrometer scale are known to be osseointegrative. The aim of this study was to evaluate the bone formation at the surface of acid etched implants modified on the micro-scale. We compared implants with a nonparticulate texture made by chemical milling (hydrofluoric acid, nitric acid) (control) with implants that had a dual acid etched (hydrofluoric acid, hydrochloric acid) microtexture surface superimposed on the primary chemically milled texture. We used an experimental joint replacement model with cylindrical titanium implants (Ti-6Al-4V) inserted paired and press-fit in cancellous tibia metaphyseal bone of eight canines for 4 weeks and evaluated by histomorphometric quantification. A significant twofold median increase was seen for bone ongrowth on the acid etched surface [median, 36.1% (interquartile range, 24.3–44.6%)] compared to the control [18.4% (15.6–20.4%)]. The percentage of fibrous tissue at the implant surface and adjacent bone was significantly less for dual acid textured implants compared with control implants. These results show that secondary roughening of titanium alloy implant surface by dual acid etching increases bone formation at the implant bone interface.

Keywords

osseointegration; surface texture; acid etching; implants; histology

INTRODUCTION

Total joint replacement is a common treatment for joints affected by osteoarthritis, rheumatoid arthritis, or trauma. Prostheses with a roughened surface inserted without cement are popular worldwide, especially for younger, more active patients. These prostheses show clinical success in general, but there seems to be a discrepancy regarding the extent of bone growth within the implant porosity.^{1,2} Early implant fixation securing stability is critical in obtaining good long-term success of total joint replacement. Rapid bone ongrowth protects the bone–implant interface against osteolysis by fluid pressure and wear particles.^{3,4} On the other hand, fibrous encapsulation, induced by excessive micromotion at the interface beyond a tolerated threshold, may hinder the integration of an implant in surrounding bone.^{5,6}

In an attempt to improve bone formation and implant fixation, various implant surface modifications have been investigated. Each technique produces a unique surface and includes physicochemical methods applying calcium phosphate coatings,⁷ biochemical methods using growth factors or cell attachment peptides,^{8–10} and morphological methods modifying implant surface area and texture. Texturing is altered by additive methods such as plasma spray or by removing some of the surface by subtractive methods such as blasting^{11,12} and chemical etching.

Surface texturing by acid etching has been studied on titanium implants *in vitro*.^{13,14} Likewise, *in vivo* studies have shown promise to enhance the potential for implant integration.^{15–21} In the dental field, texturing by acid etching has only recently been introduced, but less frequent in orthopaedics. The topography of an etched implant relies on acid mixture, etching time, temperature, and roughening prior to etching. Depending on the etching conditions, the process creates pits of depth and roughness on the micrometer scale. In general, new bone forms on a textured implant surface with a R_a of 1.0–7.0 μm .^{19,22,23} The finer surface textures are postulated to be advantageous for stimulating new bone growth onto and into porous surfaces. The surface topography obtained by acid etching can be modulated according to prior treatment. Acid etched implants show increased bone-in-contact percentage at the interface when compared with machined surfaces.¹⁵ Similar findings are described comparing etched surfaces with grit-blasted,^{16,17,19} plasma-sprayed,²⁰ and sintered²¹ titanium implants. We are unaware of prior studies that have investigated the osteoconductive effect of the titanium alloy implant with a dual acid etched (DAE) microtexture superimposed on a primary textured implant surface without the primary particulate additive or subtractive roughening.

The aim of this study is to evaluate the peri-implant bone and tissue formation at implants with two different surface configurations and to topographically describe the surfaces examined. We compare titanium alloy implants with a surface that has a primary coarser texture by a controlled chemical milling process to implants with a secondary DAE microtexture surface. Both implants undergo the primary texturing process and the microtexture is superimposed on this texture. We use a canine experimental joint replacement model with cylindrical implants inserted press-fit in cancellous tibial metaphyseal bone to represent the cancellous portion of a press-fit joint replacement implant. We quantify the peri-implant tissue formation by histomorphometric evaluation. We hypothesize that dual acid etching of a nonparticulate roughened implant surface improves the bone ongrowth at the bone–implant interface.

MATERIALS AND METHODS

Implants

Two different implant surfaces were investigated: (1) Dual acid etched (DAE) microtextured implants and (2) chemically milled textured implants serving as negative control (Control).

The test implants were cylindrical with a nominal diameter of 6 mm and a length of 12 mm. The core consisted of titanium, aluminium, and vanadium alloy (Ti6Al4V) with a machined surface (Biomet®, Warsaw, IN). The acid etched implants were manufactured by 3i (Palm Beach Gardens, FL) using the control implants done by Cyncam (ChemTex®, Houston, PA).

The control implants were acid textured by forming a random irregular pattern through repetitive masking and chemical milling. An acid resistant masking was sprayed onto the implant in a random pattern and then chemically etched at the unmasked surface in a common 30% nitric acid, 6% hydrofluoric acid solution at 43°C for approximately 4 min.

The masking was then cleaned and sprayed once again in a random pattern. The entire process was repeated for a total of three treatments.^{24,25}

A dual thermo acid etching process was performed on implants identical to the control implants.²⁶ At first the implants were immersed in aqueous hydrofluoric acid. The implants were further etched in a mixture of a dilute hydrofluoric acid and hydrochloric acid to create the surface texture. In-between each etching process, the implants were rinsed in deionized water and finally subjected to a 22.4% (w/w) nitric acid rinse at 50–60°C for 2 min, followed by 99% isopropyl alcohol for 1 min and flashed dry. The acid etched microtexture was superimposed on the central 6 mm part of the control implants with the outer edges being masked off during the procedure.

Implant surface characterization

Qualitative impression of the implant surface topography was performed by scanning electron micrographs (Camscan Maxim 2040; Camscan Electron Optics, Cambridge, UK). Quantitative roughness measurements were performed by the Danish Technological Institute, Copenhagen, with a roughness profilometer (Simicronic Surfscan, 3CS; Hommel Somicronic, France). The measured length was 5.0 mm, using an absolute/nonskid stylus. Eight measurements were performed on a titanium disk of each surface group, with a 45° rotation between each measurement. Four height parameters R_a , R_y , R_z , and R_q were used. R_a describes the arithmetic average of the deviation from the mean line over a sampling length. R_y expresses the maximum roughness depth. R_z measures the average height difference between the five highest peaks and the five lowest valleys, and R_q is the root mean square value of the profile departure.

Test animal model

The study was approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation. A paired experimental canine study was done. We used eight skeletally mature American Hound Dogs (HPR, Denver, CO) with a mean age of 11.4 months (10.9–12.5) and mean weight of 21.5 kg (20.0–22.7). The dogs were all female and bred for research purpose.

Non-weight-bearing implants were inserted bilaterally and press-fit in the extraarticular cancellous bone site perpendicular to the anterior medial plane of the proximal tibia 17 mm below the joint line (Fig. 1). The implants were inserted in a cancellous bone environment similar to that of the metaphyseal portion of a joint-replacement prostheses. The DAE textured implants were on one side, and the controls were inserted contralaterally.

Surgery was performed under general anaesthesia and under sterile conditions. Prophylactic antibiotic ceftriaxon (Rocephin; Roche Pharmaceuticals) was administered for the first 3 days. All implants were handled by the same surgeon. The implantation site was exposed through a medial anterior parapatellar approach leaving the medial collateral ligament intact. The periosteum was elevated at the implant insertion area. Using a 1.2-mm Kirschner wire as a guide, the implants were inserted in the metaphyseal bone 1.7 cm distal to the tibiofemoral joint line, perpendicular to the anterior medial plane of the proximal tibia surface. The implants were inserted press-fit as the bone bed was drilled with a cannulated drill sized 0.1 mm smaller than the outer diameter of the implant. Drilling speed was less than 2 Hz and the drill water was cooled with saline irrigation drip to avoid thermal trauma to the bone. The implants were inserted with light hammer blows. All implants exhibited primary stability. The overlying soft tissue was closed in layers. X-ray was obtained postoperatively to verify correct implant position.

All animals were allowed unrestricted weight bearing after surgery. Following a postoperative recovery period, the animals received daily exercise periods with play-time and socialization within the housing room. The animals were housed individually but socialized in groups. The daily routines in the stall followed normal circadian rhythm. The animals were fed a nutritionally complete and balanced commercial standard diet (Harlan Teklad 21% Lab Dog Diet; Harlan, Indianapolis, IN) (Protein 21%, Ca 1.28%, P 1.09%). We chose an observation period of 4 weeks, as prior studies have shown that osseointegration develops within this period in our experimental animal model.²⁷ At euthanasia the knee joint fluid was cultured, and the proximal tibiae with the implants *in situ* were removed and stored at -20°C .

Specimen preparation

Specimen preparation was done blinded. Sections were cut perpendicular to the implant axis, using a water-cooled diamond band saw and implant-based alignment post (Exact Appertebau, Nordenstedt, Germany). The 5-mm middle section was used for undecalcified histomorphometric evaluation. The specimens were dehydrated in graded ethanol (70–100%) containing 0.4% basic fuchsin (Merck, Darmstadt, Germany), followed by embedding in methylmethacrylate (Merck, Hohenbruun, Germany). The vertical section technique was applied with random rotation of the specimen blocks, and serially sectioned to 20 μm parallel to the implant axis using a microtome (Leiden Microtome KDG-95, Leiden, Holland). Four serial specimens were obtained from the center of the implant. After specimen sectioning, the surface was counterstained with 2% light green (BDH Laboratory Supplies, Poole, England). The staining technique makes it possible to visualize mineralized bone, fibrous tissue, and marrow-like tissue.

Histological analysis

Bone ongrowth at implant surface and peri-implant bone volume fraction was quantified by static histomorphometry. Computer-assisted histomorphometry was performed blinded in random specimen order with light microscope (Olympus BX51 TF, Olympus Denmark) and image analysis system (CAST-Grid, Version 2.1.4, Olympus Denmark) at a magnification 400 $\times$. Stereological methods were applied using systematic uniform random sampling to obtain unbiased estimates.²⁸ Tissue ongrowth was defined as tissue directly at the implant surface, and was determined using the linear intercept technique with random disposed sine weighted lines. Peri-implant tissue volume fraction was achieved using point counting in separate zones of 0–100 and 100–500 μm from the implant surface with random point disposition. Each zone was evaluated in independent counting sessions. Bone ongrowth is expressed as tissue percentage and peri-implant density as tissue volume percentage.

Statistical analysis

STATA Intercooled 8.2 statistical software was used. Differences between the two implant types were analyzed using the Wilcoxon sign ranks test for paired comparisons. A nonparametric analysis was applied since differences between pairs did not follow a normal distribution. Two tailed *p*-values less than 0.05 were considered statistically significant.

RESULTS

Surface topography

Scanning electron micrographs of the implant surfaces revealed the textured surface of the DAE implants on the micrometer scale (Fig. 2). A honeycomb texture with sharp-edged pits made up the surface. The surface of the control implants had a smoother surface texture. The results of the roughness measurements are shown in Table I.

Surgery

All animals were ambulatory the same day of surgery and fully weight bearing after 2 days of recovery. There were no postoperative complications. No animals lost weight or were excluded during the observation period. No clinical infection was observed at euthanasia and no bacterial growth was detected in joint fluid culture. All implants revealed clinically firm anchorage in bone.

Histomorphometry

At the implant bone interface, a significant increase in bone contact was observed for the DAE implants (median, 36.2%; interquartile range, 24.3–44.6%) compared with the control implants (median, 18.4%; interquartile range, 15.6–20.4%) ($p = 0.01$) (Fig. 3). The percentage of fibrous tissue at the implant surface was significantly less for dual acid-textured implants (median, 0.0%; interquartile range, 0.0–0.0%) compared with control implants (median, 2.9%; interquartile range, 7.6–19.8%) ($p = 0.02$).

In the 0–100- μm concentric zone adjacent to the implant surface, the dual acid textured implants had higher bone volume fraction (median, 35.1%; interquartile range, 28.6–41.0%) compared with the controls (median, 30.4%; interquartile range, 26.3–34.1%), but this was not significant ($p = 0.26$).

In the 100–500- μm concentric zones, most distant to the implant surface, no significant differences were found in bone volume fraction between the two implant surfaces, but the percentage of fibrous tissue was significantly less for the DAE implants (median, 0.1%; interquartile range, 0.0–0.3%) compared with control implants (median, 0.9%; interquartile range, 0.3–3.5%) ($p = 0.03$) (Fig. 3).

DISCUSSION

Chemical texturing is a method of surface roughening through acid etching. This method has advantages compared with current methods of roughening such as grit blasting, plasma spraying, and beads. As the production process does not stress the adjacent material, there is no risk of flaking or delamination and the process does not leave particles of grit.

The aim of this study was to determine whether a positive bone response is observed around a titanium alloy implant with a surface that is textured by dual acid etching superimposed on a surface primarily roughened by a chemical milling process. We used the chemically milled implant as a control implant. This was to ensure that it actually was the dual acid etching process that stimulates bone ongrowth and not the preceding chemical milling process. We used an experimental canine model being advantageous in resembling the clinical practice of cementless fixation in the cancellous bony environment with total joint prostheses in humans. The canine was chosen as the animal testing model, since its bone architecture most closely resembles human bone.²⁹ The implants were inserted press-fit with direct contact between the implant surface and the host bone. An environment abundant in cancellous bone in the proximal tibia was chosen. We evaluated the bone healing at the implant surface during the early stages of bone regeneration and chose an observation period of 4 weeks based on previous studies.²⁷ By inserting implants contralaterally the two different surface textures could be compared in a paired design within each animal. This reduces the influence of interindividual biological factors on bone healing. Although the study was limited by the implants being non-weight bearing, the conditions of this study mirror the situation of bone weight transmission in periarticular bone in the weeks after implantation of uncemented prostheses. The effect on mechanical fixation is not addressed since the chemical milling process did not allow suitable length of implant for mechanical testing in

addition to histomorphometrical evaluation. We hypothesized that bone ongrowth would be higher on a DAE surface compared with the chemically milled control implant.

We found that surface texturing with dual acid etching had a bone stimulating effect directly at the interface. The total bone-to-implant contact was increased significantly by 96%. All the etched implants showed more bone ongrowth than their contralateral control implants. This is encouraging as it suggests that the dual acid etching process can improve osteoconductivity at the implant surface. Just as importantly, we observed a significant reduction in fibrous tissue in all three zones of interest. From a clinical view point, fibrous tissue is unwanted on the implant interface. It may prevent bone ingrowth and result in micromotion of the implant as a result of less firm-anchorage. Clinically loosened implants are typically surrounded by dense fibrous tissue.

Acid etching of titanium implants has been examined *in vivo* in orthopaedic implant device models.^{15,17,19,21} No studies are available on the osseointegration of implant surfaces with etching on a primary chemically roughened surface without the potential coating-debonding by embedding of surface contaminants (e.g. grit particles), or by the addition of particulate matter (e.g. plasma spray coating, beads). Other studies report up to 70% increase in bone contact with orthopaedic implants etched secondary to a particulate coarser surface texture. This is consistent with our findings. Hacking et al.²¹ evaluated the bone growth on cylindrical titanium implants with an acid microtexturing superimposed on the surface of sintered titanium beads in a canine study with non-weight-bearing implants inserted intramedullarily in the femur. After 12 weeks observation, a 60% enhancement of bone ingrowth with the acid etched implants was observed. Buser et al.¹⁵ revealed a 57% bone in contact for sandblasted acid etched implants compared with 33% for sandblasted without etching in a study with miniature pigs having inserted titanium cylindrical implants press-fit in metaphyseal bone for 6 weeks. Wong et al.¹⁹ studied the bone integration of implants made of titanium and various alloys with surfaces modified by fine blasting, rough blasting, rough blasting/acid etching, and hydroxyapatite coating. The implants were inserted press-fit in metaphyseal cancellous bone in miniature pigs for 12 weeks. The bone surface coverage of the HA-coated implants was about twice the average surface coverage by bone of the other all-metal groups, but there was no significant difference among any of the all-metal groups. This is not in accordance with the bone stimulatory effect we found for the DAE implant surface. The explanation for this might be that the osseointegration of implants depends on a number of factors such as implant design and topography, type of metal, test animal, implant bed, surgical procedure, loading condition, and hormonal factors. These various factors may be ascribed to deviation from our finding.

In this study, we found that DAE implants reveal a microtextured surface. Topographic properties of implant surfaces relate to the degree of roughness. *In vitro* studies show that smooth titanium surfaces (arithmetical mean roughness $R_a < 0.5 \mu\text{m}$) are not able to stimulate osteoblastic activity, but roughened surfaces can (R_a range, 1–7 μm).^{30–35} Similar *in vivo* and clinical studies demonstrate the formation of new bone on textured surfaces (R_a range, 1–7 μm), but formation of a fibrous membrane at the interface of a stable smooth implant surface ($R_a < 0.4 \mu\text{m}$).^{19,22,23} Above a threshold ($R_a \approx 0.5 \mu\text{m}$) the more delicate microtextured surfaces (R_a range, 1–2 μm) develop more rapidly and greater bone coverage compared with the more rough textures (R_a range, 4–5 μm).^{12,19} We found that the etching procedure produced a characteristic surface structure when viewed with the scanning electron microscopy. The secondary etched implants had a more distinct rough surface than the primary chemically milled implants. This structural difference was reflected in our finding that textured implants showed a stronger bone response than our controls and those irregularities down to 2–4 μm may be invaded by bone. In this study, we only found subtle differences in average roughness between the primary chemically milled implants and the

secondary etched implants. Osteoblasts are known to be sensitive to delicate differences in surface roughness and surface chemistry.^{14,36} It is unclear from this study whether the acid etching process is osseointegrative by the physically topographic roughness alteration of the surface we observed in the scanning electron microscopy, or if the etching process alters the electrochemical properties of the implant surface.

Acid etching has in addition to its use in orthopaedic implant devices been used as a method in modifying the surface of dental implants. When inserted in the mandible and tibia, these threaded implants show increased bone-in-contact percentage at the interface when compared with machined,^{15,37} grit-blasted,¹⁶ and plasma-sprayed surfaces.²⁰

CONCLUSION

In conclusion, this study showed promising results on the improved osseointegrative effect of an orthopaedic titanium alloy implant surface textured by a dual acid etching process. In clinical practice only a minor part of the implant inserted uncemented press-fit is in direct contact with bone early after implantation. Therefore, further studies with implants inserted in a gap and weight-bearing conditions are necessary to evaluate the osseointegrative effect of surfaces microtextured by the dual acid etching process. Also the osseointegrative causality of the microtextured surface needs further attention, and the mechanical consequences of improved bone ongrowth should be identified.

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Figure 1.
Test implant device of titanium alloy (Ti6Al4V) (6×12 mm) inserted press-fit and unloaded in cancellous bone of the canine proximal tibia.

Figure 2. Scanning electron micrograph of (a, b) control implant textured by chemical milling and (c, d) implant textured by dual acid etching. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 3.

Histomorphometry. Dual acid etched textured implant compared with control implant inserted press-fit in proximal tibia cancellous bone. Volume fraction of bone, marrow-like, and fibrous tissue at implant surface (top), concentric zone 0–100 µm (middle), and 100–500 µm (bottom). Paired data are connected by dotted line. Median value indicated by horizontal line. $N=8$. DAE = dual acid etched surface texture; Control = surface texture by chemical milling.

TABLE I

Mean Surface Roughness (μm) of Implants with a Chemically Milled Texture and Dual Acid Textured Surface, Characterized by Height and Spatial Parameters

Surface Treatment	Control	DAE
R_a	32.1 (6.2)	37.0 (5.3)
R_z	153.9 (19.1)	166.7 (21.1)
R_q	39.2 (6.9)	44.4 (5.8)
R_v	68.2 (8.8)	75.4 (14.1)

R_a = arithmetic mean of the deviations of the roughness profile from the midline; R_y = maximum roughness depth; R_z = average height difference between the five highest peaks and the five lowest valleys; R_q = the root mean square value of the profile departure. DAE = dual acid etched surface texture; Control = surface textured by chemical milling. Values within parentheses are standard deviations.