Influence of furrow width on the stability of titanium implants. A study in the rabbit

ABSTRACT

Aim Previous rabbit studies demonstrated higher affinity for bone formation at implant threads with furrows to threads without furrows. The present animal study was undertaken to study the bone tissue response and stability of oxidized titanium implants with 80, 110 and 160 μm wide furrows added on one thread flank.

Materials and methods Ninety-six (96) threaded titanium implants, 3.75 mm in diameter and 7 mm long (TiUnite™, MKIII, Nobel Biocare AB, Gothenburg, Sweden), were manufactured with 70 μm deep and either 80 (S0), 110 (S1) or 160 (S2) μm wide furrows or no furrows (controls). The implants were installed in the distal femoral condyle and the tibial metaphysis of 12 rabbits. Six weeks later the implants were subjected to resonance frequency analysis (RFA) and removal torque (RTQ) tests, after ground sections were manufactured for light microscopy.

Results A significantly increased (22%) stability of S1 implants in femoral sites compared with control implants was found. RFA showed no significant differences between test and control implants. Histology revealed more frequent bone fill of furrows with decreased width in parallel with increased frequency of fracture of the bone at the furrow entrance as opposed to a separation at the interface.

Conclusion The present study demonstrated an increased and maximum rotational stability of oxidized titanium implants with a 110 μm wide furrow on one thread flank compared with control implants without a furrow. The observed increased stability can be explained by fracture of the bone at the entrance of the furrow as opposed to a separation at the interface as seen at the wider furrows and at control implants.

INTRODUCTION

Absence of mobility at the bone-titanium implant interface is regarded as detrimental to achieve and maintain successful bone integration of dental implants. Primary implant stability is determined by factors such as bone density, surgical technique and implant design and can therefore be influenced by the surgeon (1-3). Secondary stability is in addition to primary stability also depending on the tissue response to the implant surface during healing (3, 4). Studies have demonstrated positive effects of surface modification of titanium implants on integration kinetics and stability. For instance, higher degrees and more rapid formation of direct bone-implant contacts (5-8) as well as higher resistance to shear forces (9-12) have been reported when comparing modified and non-modified titanium surfaces. Although the influence of surface chemistry factors should not be underestimated, the positive outcome is probably an effect of modified topography at the micrometer level. Plasma-spraying, blasting, acid-etching and anodic oxidation, and combination of techniques, usually results in structures on the micrometer level and a larger surface area (13), which in turn offers increased mechanical retention due to bone ingrowth (14). This may explain higher removal torques for rough surfaced implants compared to more smooth ones. However, it is also evident that the pattern of bone integration may differ between surfaces. It is generally anticipated that turned, relatively smooth, implant surfaces are integrated by so called distance osteogenesis, i.e. bone growth from the osteotomy towards the implant surface (15), while it has been speculated that some surface modified implants also show bone formation directly on the implant surface, so called, contact osteogenesis (16, 17). The conception of contact osteogenesis entails migration and differentiation of osteogenic cells and subsequent bone formation directly on a surface not in contact.
with surrounding bone. The exact mechanisms are not known but retention of the initial blood clot and fibrin network to enable cell migration to the interface area and aggregation of platelets has been pointed out as important (18). Perhaps it is more likely that the integration process proceeds as bone formation along the implant surface from a point initially in contact with the surrounding bone, i.e. osteoconduction. In any case, the modified surface topography may result in mechanical forces at the cellular level that stimulates formation of gradients of chemotactic and other molecules, which drive cell migration towards or along a surface. In vitro work has shown that osteoblasts seem to attach stronger at rough than at smooth surfaces (19), and therefore, the mechanical forces on the cells may be different at the two surfaces.

In a previous rabbit study, the tissue response and stability of threaded oxidized titanium implants with either a 110 or 200 µm wide furrow added to one thread flank were studied (20). Interestingly, the histological analyses after six weeks revealed an affinity for bone formation within the furrows when compared to surfaces without a furrow. In that model most of the implants threads were located in bone marrow tissue and without primary bone contacts and the results indicated that the furrow provided an environment suitable for bone formation, possibly due to mechanical forces, blood clot retention, the presence of chemotactic agents etc. It seemed that the bone tissue within the furrows originated from the cortical passage and followed the path of the furrow. Removal torque tests showed a 30% increase of stability for 110 µm wide furrows compared with control implants without a furrow. The wider furrow showed no such increase in stability. It was speculated that the difference was due to the location of the bone fracture, which was above the entrance of the 110 µm wide furrows and at the bone-implant interface at the 200 µm wide furrows. However, the hypothesis could not be verified in that study since no histology after removal torque testing was conducted.

The aim of the present study was to further evaluate the influence of macroscopic furrows on the stability of screw-shaped titanium implants with an oxidized surface as measured with resonance frequency analysis (RFA) and RTQ tests. In this study 80, 110 and 160 µm wide and 70 µm deep furrows were investigated. In addition, the bone-implant interface was histologically evaluated after RTQ testing.

**MATERIALS AND METHODS**

**Animals and anaesthesia**

Twelve female New Zealand white rabbits, at least 6 months old, were used in the study. The animals were kept free in a purpose-designed room and were fed ad libitum with water and standard laboratory animal diet and carrots. Prior to surgery, the animals were given general anaesthesia by an intramuscular injection of flunisan and fentanyl (Hynpom, Janssen Pharmaceutica, Brussels, Belgium) 0.2mg/kg and intraperitoneal injection of diazepam (Stesolid, Dumex, Copenhagen, Denmark) 1.5 mg/kg body weight. Additional Hypnorm was added when needed. Local anaesthesia was given using 1 ml of 2.0% lidocain/epinephrine solution (Astra AB, Södertälje, Sweden). After surgery the animals were kept in separate cages until healing of the wounds (1-2 weeks) and then released to the purpose-designed room until termination. Postoperatively, the animals were given antibiotics (Intenpencillin 2.250.000 IE/5 ml, 0.1 lm/kg body weight, LEO, Helsingborg, Sweden) and analgesics (Temgesic 0.05mg/kg, Reckitt and Colman, NJ, USA) as single intramuscular injections for three days. The study was approved by the local committee for animal research.

**Implants and surface analysis**

Ninety-six (96) threaded titanium implants, 3.75 mm in diameter and 7 mm long (TiUnite, MKIII, Nobel Biocare AB, Gothenburg, Sweden), were manufactured without any apical tapping features. Three groups of test implants had a single furrow positioned at the center of the inferior thread flank, i.e. facing the head of the implant. The furrows were 70 µm deep and either 80 (S0), 110 (S1) or 160 (S2) µm wide (Fig. 1 a-c). Implants without furrows were used as controls (C). All implants were subjected to surface modification by anodic oxidation (TiUnite, Nobel Biocare AB, Gothenburg, Sweden) as described elsewhere. The implants had a special internal feature, which allowed for using a special connector (Stragrip, Nobel Biocare AB, Gothenburg, Sweden) to ensure firm grip when placing the implants and when performing removal torque measurements (see below.)

Topographical analysis was performed using an optical interferometry (MicroXAM™, PhaseShift, Tucson, USA) with measurement area of 200 x 260 µm (50X objective, zoom factor 0.625) and the errors of form were removed with a digital Gaussian filter (size 50x50 µm). Images from the thread top, valley, inferior thread flank and furrow were obtained at three different levels of implants: top, middle and bottom. 36 areas per specimen were analysed and the following 3D parameters were calculated (Table 1).

\[ S_h(\mu m) = \text{the arithmetic average height deviation from a mean plane. } \]

\[ S_v(\mu m^{-1}) = \text{the density of summits. } \]

\[ S_d(\%) = \text{the developed surface ratio. } \]

\[ S_{cr} = \text{core fluid retention index. } \]
Mathematical descriptions of the parameters can be found in the literature (21).

Surgery
Both tibial metaphyses and the distal femoral condyles were used as implantation sites. Four implants, one from each group, were inserted in tibial and femoral sites, respectively, according to a rotational scheme. Thus, each animal received a total of 8 implants. The experimental areas were exposed via a skin incision medial to the knee-joint and separate incisions through fascia and periosteum above each site. Two holes were drilled through one cortical layer in each tibial metaphysis and femoral condyle during generous cooling by saline. The implants were placed after preparation with 2.0 mm and 3.0 mm twist drills followed by screw tapping. The implants were inserted with a torque of 30 Ncm. No countersink drill was used, i.e. the implant head rested on the cortical bone. All implants were placed monocortically. The fascia-periosteal flap and the skin were closed in separate layers with resorbable sutures. After a healing period of 6 weeks the animals were anesthetized for stability measurements (se below). The animals were then killed by an overdose of pentobarbital (Mebumal, ACO Läkemedel, Solna, Sweden).

Resonance frequency analysis
All implants were subjected to resonance frequency analysis measurements at installation and after 6 weeks of healing using an Osstell instrument (Integration diagnostics, Göteborg, Sweden). A resonance frequency transducer was connected to the implant perpendicular to the long axis of the
tibia and femur and an ISQ (Implants Stability Quotient) value was recorded.

**Removal torque testing**

The resistance to removal torque was tested with an electrical torque transducer consisting of a torsion rod mounted on a stable metal framework. The bone with implants was fixed in a vice to ensure application of perpendicular load during testing. The rod was connected to each implant with the connector described above. An electronic motor ramped the torque to a maximum value, which was registered and stored by a micro processor. At the point of interfacial failure between the bone and the implant, the peak force dropped and a slight rotational movement of the implants was observed.

**Histology**

All implants were retrieved for histology after the stability, i.e. RTQ and RFA, measurements. The implants and surrounding bone tissues were removed in blocks and fixed by immersion in 4% buffered formaldehyde. The specimens were dehydrated in graded series of ethanol an embedded in light curing plastic resin (Technovit 7200 VCL, Kulzer, Friedrichsdorf, Germany). Sections were taken through the longitudinal axis of each implant by sawing and grinding (Exakt Apparatebau, Norderstedt, Germany). The sections, about 10 μm thick, were stained with toluidine blue and 1% pyronin-G. Examinations were performed by a Nikon Eclipse 80i microscope (Teknoptik AB, Huddinge, Sweden) equipped with an Easy Image 2000 system (Teknoptik AB, Huddinge, Sweden) for morphometrical measurements.

The histometric evaluation comprised the following.

> Measurements of the thickness of the supporting bone. This was defined as the height of the bone tissue projected towards the implant surface from the surroundings (Fig. 2a, b).
> Quantification of the proportion of furrows with and without bone tissue.
> Quantification of the proportion of furrows with bone showing fracture of the bone at the entrance of the furrow.
> Quantification of the proportion of furrows with bone showing no signs of fracture but a separation between bone and the implant surface.

**Statistics**

The Wilcoxon Signed Rank and Spearman Rank Correlation tests were used for statistical evaluations of the material. A difference was considered when p < 0.05.

**RESULTS**

The animals responded well to surgery and no complications were observed during the postoperative healing period. All 96 implants, 24 from each group were successfully subjected to RTQ and RFA measurements.

A typical histological section of a tibial site comprised the implant and the triangular shaped tibial cortex with bone marrow inside (Fig. 2a). Bone trabeculae from the lateral part of the tibia were occasionally seen projecting towards the implant surface. The implant was secured with one or two threads in the cortical passage, while the remaining threads were situated in the marrow cavity. Rupture
of the interface was evident due to the destructive RTQ test. Nevertheless, bone formation was evident at and near the implant surface. The cortical bone showed signs of remodeling at both periosteal and endosteal surfaces. The femoral sites consisted of an outer cortical layer and various amounts of cancellous bone and bone marrow (Fig. 2b). Most of the implant surface was involved with trabecular bone. Displaced bone fragments from the drilling procedure could be observed apically. Bone formation and remodeling was evident at and near the implant surface. Also the displaced bone fragments showed bone formation on the surface. Morphometric evaluation showed an average of 1.4 mm (SD 0.3) of supporting bone at tibial implants. The femoral implants were supported by 4.0 mm (SD 1.6) bone. The anterior sites in the femur showed somewhat more supporting bone than the posterior sites, while there were no differences in the tibial sites. Both tibial and femoral implants showed increased degree of bone fill of the furrows with decreasing width (Fig. 3, 4). The fracture made by the RTQ measurements was more often located within the bone at the furrow entrance for the S0 and S1 implants (Fig. 5 a-b), whereas the fracture location at the S2 implants was more often located at the bone-implant interface (Fig. 5c). Statistical analysis showed a significant correlation between decreased furrow size and bone fill as well as the number of fractures at the furrow entrance for both tibial and femoral implants. Higher RTQ values were in general measured in...
femoral than in tibial sites. There was a correlation between RTQ value and supporting bone height in both femoral and tibial sites. The femoral implants showed statistically significant higher values for S1 but not for S0 and S2 implants compared to controls (Table 2). The mean percentage difference between test and control implants were 22.0% (SD 28.2) for the S1 implants, 8.5% (SD 19.3) for S0 implants and -3.7% (SD 22.2) for the S2 group. For tibial implants there were no statistically significant differences between test and control implants (Table 3). The mean percentage difference between test and control implants were 19.6% (SD 50.2) for S2 implants, 0.3% (SD 36.5) for S1 implants and -1.9% (SD 28) for S0 implants. There were no differences between anterior and posterior implant sites.

RFA measurements showed a numerical increase of the ISQ value for all implant groups. There were no statistically significant differences when comparing the primary stability, secondary stability or change of stability for test and control implants (Table 4 and 5).

**DISCUSSION**

The present experimental study was undertaken to evaluate the influence of various widths of macroscopic furrows on the stability of screw-shaped titanium implants as measured with RTQ and RFA. The results from RTQ measurements showed that 110 µm wide furrows (S1 implants) were statistically significant more stable than control implants without a furrow in femoral bone, about 22.0% (p<0.05). This is in line with a previous study using the same animal model (20). However, in the present study no differences could be statistically verified in tibial bone, whilst the previous study showed an increase of 30% for S1 implants in this location. Morphological differences between tibial and femoral site can explain the higher RTQ measurements seen for femoral implants in the present study, and also the difference between tibia implants in this and the previous study. Only one or two threads were located in the cortical bone of tibia. Small differences in the extension of the furrow into the thin cortical bone can result in dramatic variations in the measured RTQ values. Thus, the values are strongly dependent on cortical bone thickness and vertical implant position in tibia.

The femoral condyle consists of both cortical and underlying trabecular bone whilst the tibia has a thin cortical layer only. The morphometrical measurements showed on average about 3 times more of supporting bone for femoral compared with...
For narrower furrows, the number of fractures follows. However, the decreasing furrow width should have been expected a linear correlation between these parameters and furrow. In parallel, an increased fill with bone was furrow more often with decreased width of the bone fractured at and outside the entrance of the present study confirmed the hypothesis that the bone fractured at and outside the entrance decreases. If the force required fracturing bone is larger than the force required to fracture the interface, then the RTQ values should increase. However, as the furrows become narrower, the forces required to fracture the bone at the furrow entrance decreases, and decreased RTQ values should be expected and approach the values for the control implants without furrows. Therefore, the curve of RTQ values as a function of furrow width must have a maximum, which seems to peak close to the 110 µm wide furrow (S1) in this experiment.

The present study confirmed our previous findings that bone formation occurred more often within the furrow than on surfaces without a furrow which indicated that the furrow microenvironment promoted bone formation. Interestingly, the morphometrical analyses also revealed an increased affinity for bone formation with decreased width of the furrow. The cell and tissue response to furrowed implant surfaces has been studied in numerous in vitro experiments (19) and a rabbit model (25). From studies in cell cultures the term contact guidance has been coined based on the observation that cells become oriented and migrate in direction with the furrows on a substrate. The furrows of the present study were much larger than typically used in the above mentioned experiments and it seems unlikely that the differences in bone fill between the narrow and the wider furrows can be explained by contact guidance only. Concentration and gradients of chemotactic and other agents from the healing process is a more plausible explanation. Moreover, it is also known that implant shape can have an influence on the bone tissue response to a biomaterial due to constraining of cell populations in to a limited space which seems to favor differentiation and bone formation. In vivo studies including early observations may aid in the understanding of the mechanisms for preferential bone growth within the narrower furrows. Such studies are underway.

In general, the surface analyses showed a similar topography for all implants and between different parts of the implants. The surface inside the furrow of S1 implants showed a slightly higher Sa and Sdr value indicating a higher roughness which may have contributed to the results. However, since only one implant from each group was measured, this difference could no be statistically verified and may thus depend on variations between implants.

In conclusion, the present investigation demonstrated a maximum removal torque value for implants with a 110 µm wide furrow added to one thread flank when placed in femoral bone. The maximum RTQ value was significantly different from tibial implants. This means that more bone was involved with femoral implants and their furrows, which was evident as higher RTQ values than in the tibia when comparing control implants. Although the supporting bone was 3 times higher, the RTQ value was only 1.6 times higher for femoral implants. This is probably reflecting the biomechanical properties of the trabecular bone at the femoral site. The fact that anterior femoral sites showed more supporting bone than posterior sites, but there were no differences in RTQ values, support this notion. Previous work using RTQ tests and histology have demonstrated a correlation between the amount of cortical bone and peak RTQ values (22-24). Moreover, since two implants were placed in each femur and that the cancellous bone decreases with medial direction, the amount of supporting bone varied depending on implant position. This was seen as a high standard deviation for supporting bone in the distal femoral condyle.

RFA measurements revealed no differences between test and control implants with regard to primary or secondary stability. It is interesting to note that there were no obvious differences in ISQ values between tibial and femoral sites in spite of the different morphologies and degree of supporting bone. The RFA technique measures lateral stability (4) and studies have shown that bone density is one determinant for primary stability provided that the same implant design and drilling technique is used (1-3). Although the femoral sites contain more bone, the density of the marginal bone appears similar during drilling. The applied frequency is probably effectively damped over 1-2 mm in dense bone, which may explain the lack of differences.

In a previous study on implants with macroscopic furrows it was suggested that the higher RTQ values seen for 110 µm compared with 200 µm wide furrows could be explained by different patterns of bone fracture/separation at the bone-implant interface (20). The histological analysis after RTQ tests in the present study confirmed the hypothesis that the furrow microenvironment within bone at the furrow entrance increases and the interface fractures decreases. If the force required fracturing bone is larger than the force required to fracture the interface, then the RTQ values should increase. However, as the furrows become narrower, the forces required to fracture the bone at the furrow entrance decreases, and decreased RTQ values should be expected and approach the values for the control implants without furrows. Therefore, the curve of RTQ values as a function of furrow width must have a maximum, which seems to peak close to the 110 µm wide furrow (S1) in this experiment.

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RTQ values measured for the wider and narrower furrows, respectively. An explanation for the maximum value was based on the observation that furrows of the bone above the entrance of the furrow were more frequent for narrow than for wide furrows. The histological analyses revealed a not previously observed affinity to bone formation within furrows with decreased width.

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