Influence of Micro Threads Alteration on Osseointegration and Primary Stability of Implants: An FEA and In Vivo Analysis in Rabbits

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ABSTRACT

Purpose: To describe the early bone tissue response to implants with and without micro threads designed to the full length of an oxidized titanium implant.

Materials and Methods: A pair of two-dimensional finite element models was designed using a computer aided three-dimensional interactive application files of an implant model with micro threads in between macro threads and one without micro threads. Oxidized titanium implants with (test implants $n = 20$) and without (control implants $n = 20$) micro thread were prepared. A total of 12 rabbits were used and each received four implants. Insertion torque while implant placement and removal torque analysis after 4 weeks was performed in nine rabbits, and histomorphometric analysis in three rabbits, respectively.

Results: Finite element analysis showed less stress accumulation in test implant models with 31Mpa when compared with 62.2 Mpa in control implant model. Insertion and removal torque analysis did not show any statistical significance between the two implant designs. At 4 weeks, there was a significant difference between the two groups in the percentage of new bone volume and bone-to-implant contact in the femur ($p < .05$); however, not in the tibia.

Conclusions: The effect of micro threads was prominent in the femur suggesting that micro threads promote bone formation. The stress distribution supported by the micro threads was especially effective in the cancellous bone.

KEY WORDS: animal study, implant design, implant stability, osseointegration

INTRODUCTION

The term *osseointegration* refers to the process whereby alloplastic materials (e.g., dental implants) and bone are bound in a rigid, clinically asymptomatic union that withstands functional loading.[1] Dental implant primary stability has been demonstrated to be a key factor for implant survival.[2] Primary mechanical stability is directly related to the quality and quantity of bone at the recipient site, the type of implant used and the surgical technique used to place the implant.[2–4]

Studies have indicated that various surface characteristics, such as surface composition, roughness, topography, and energy, play a major role during the initial phases of bone integration to the implant.[3–5] Macro irregularities such as grooves and pores of various dimensions, which are considered as the parameters of implant design, have been introduced to implant threads in order to enhance the initial bone contact, increase the surface area, and thus dissipate interfacial stress.[6–9] Many studies have also been reported that, along with the thread designs, modification of the implant surface by anodic oxidation, which is one of the several surface treatment methods, results in a superior bone response to that achieved with a turned surface.[10–14] Various in vitro studies have been documented in literature about the cell migration, on the surface with grooved structures and also influence bone deposition around

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these implants.[15–22] Thus, indicating that macro grooves and surface irregularities play a definitive role in enhancing osseointegration. In our previous study, a prototype implant with micro threads in between macro threads was designed with turned surface; results showed that micro threads designed in between the macro threads promoted early bone stimulation in the femur.[23] Hence, in order to further enhance osseointegration, the present study was aimed to evaluate the early bone tissue response to micro threads designed to the full length of the oxidized titanium implant between the macro threads, by reducing the depth of the macro threads, thus increasing the initial contact of the micro threads with the bone. In addition, oxidized surface was used to make the implant surface isotropic in this present study.[24] Early bone growth was evaluated using histomorphometric analysis of the implant prototype in rabbits, and insertion and removal torque (RTQ) analysis was performed to evaluate the primary stability of the prototype implants. A finite element analysis was performed on prototype test and control implant model to evaluate the pattern of stress dissipation through the micro threads designed in between the macro thread.

MATERIALS AND METHODS

Finite Element Analysis

Pair of two-dimensional models, were designed using a computer aided three-dimensional interactive application (CATIA), a computer-aided designing (CAD) modeling software, and transferred in an initial graphics exchange specification format to ANSYS 12.1 (Ansys, Inc., Houston, PA, USA). One model consists of a prototype dental implant with macro threads at equal intervals with micro threads in between these macro threads. The other model consists of only macro threads at equal intervals with no micro threads in between them. Both the implant models were of 4 mm in diameter and 12 mm in length. The implant models were assumed to be embedded in homogenous cortical bone. The cortical bone was simulated as a bilinear material with no hardening effect and the implants were simulated with bilinear according to respectively material data in Table 1. The implant bone interface was simulated as frictionless connection, and the bone was assumed to be fixed at the lateral borders. Booth models were vertically loaded with a static load of 100N.

TABLE 1 Mechanical Properties for the Finite Element Model

Material Properties	Cortical Bone (Mechanical Properties of Cortical Bone Donald T Reilly J Bone Joint Surgery 1974 1001–1022)	Pure Titanium Gr 4 ASTMF67
Young's modules (Gpa)	18	105
Poisson's ratio	0.3	0.36
Yield (Mpa)	120	483
Tangent modules (Mpa)	0.1*	3,000†

*Simulation of no hardening effect.
†Simulation of hardening effect.

Implants and Surface Analysis

Based on the CAD model designs and the finite element analysis of these models, two sets of oxidized surfaced prototype titanium implants (4 mm in diameter and 8 mm in length, grade 4 titanium) were prepared for the study. The control implants possessed V-shaped macro threads at regular pitch intervals of 2.5 mm, with 60^0 thread angulation and thread depth of 0.4 mm, as compared with 0.5 mm in our previous study.[25] V-shaped micro threads of 0.26 mm in pitch-height with 0.1 to 0.2 mm of thread depth, similar to our previous study implants were added in between the macro threads for the test group implants.[25]

Topographical analysis was performed using optical interferometry (MicroXam™, Phaseshift, Tucson, AZ, USA). The procedure followed is explained in our previous study.[25]

Animals and Anesthesia

Twelve adult Swedish lop-eared rabbits (weight 3.1 to 5.4 kg) were used in the study. Prior to surgery, the animals were given general anesthesia by an intramuscular injection of a mixture of 0.15 mg mL/kg medetomidine (1 mg/mL dorimitor; Orion Pharma AB Sollentuna, Sweden) and 0.35 mL/kg ketamine hydrochloride (50 mg/mL ketalar; Pfizer AB, Sollentuna, Sweden). Lidocaine hydrochloride Xylocaine; AstraZeneca AB, Södertälje, Sweden) was administrated as the local anesthetic at each insertion site at a dose of 1 mL. The study was approved by the Malmö/Lund regional animal ethics committee.

Surgical Procedure and Insertion Torque Quotient (ITQ)

With a total of 48 prototype implants of 24 each, test and control were placed contralaterally in left and right legs of the rabbit, one in the proximal tibia and one in the distal condyle of the femur in each hind leg. The experimental areas were exposed via a skin incision medial to the knee-joint and separate incisions through fascia and periosteum above each site. Osteotomy was prepared using 2 mm and 3 mm twist drills during generous cooling by saline. The osteotomy sites were pre-tapped at 35 rpm. The fascia, periosteal flap, and the skin were closed in separate layers with resorbable sutures (Vicryl 4.0; Johnson & Johnson/Ethicon, Somerville, NJ, USA). Postoperative analgesic for 3 days were given. At the time of insertion, ITQ (Ncm) was measured in 9 rabbits out of 12, using a specially designed device (Elcomed, W&H, Milano, Italy). After 4 weeks, all the rabbits were sacrificed with an overdose (60 mg/mL) of Pentobarbitaluatrium (Apoteksbolaget AB, Stockholm, Sweden). The three rabbits, which were not used to measure ITQ, were also sacrificed and the specimens were prepared for histomorphometric analysis.

RTQ Analysis and Tissue Processing

After 4 weeks, all the implants of the nine rabbits in which ITQ was measured were subjected to RTQ test using an electronic device, with a special connector. A device used for many years as a standard technique and is described in our previous publications.[23,24] The instrument was connected to the implants, and a fixed rotation rate of reverse torque was applied until failure of the bone-implant interface occurred. The peak values of resistance to reverse torque rotation were recorded Ncm. To calculate for the initial offset generated by the RTQ device, 3 Ncm was subtracted from each obtained value.

All the implants from the three rabbits planned for histomorphometric evaluation were retrieved en bloc, surrounded by bone and fixed by immersion in 4% buffered formaldehyde. Later the specimens were dehydrated in a graded series of ethanol and embedded in light curing resin (Tecnovit 7200VCL, Kulzer, Frcedrichsdorf, Germany). Sections were taken through the longitudinal axis of each implant by sawing and grinding (Exakt apparatebau, Norderstedt, Germany). The sections were about 10 μm thick and were stained with toluidine blue and 1% pyronin-G. Examinations were performed in a Nikon Eclipse 80i light microscope (Teknooptek AB, Huddinge, Sweden), using $\times 1.8 \times 100$ objectives for descriptive evaluation and morphometrical measurements. The qualitative analysis aimed at describing the early bone formation events at the macro and in particular the micro threads. The histometric evaluation comprised of measurements of the degree of bone-implant contacts and bone volume in a specified area, along the implant threads.

Statistical Analysis

Descriptive data of measured parameters were used for the different time points. The Wilcoxon rank sign was used on pooled data from the tibia and femur of control and the test implants, and a difference considered if $p < .05$.

RESULTS

Finite Element Analysis

Stress and strain in the bone around the implants were calculated using von Mises stress and strain values. The stress levels were in the range was 0.3 Mpa to 79 Mpa in the bone for the control implant model, where as it was in the range of 0.3 Mpa to 41 Mpa for the test implant model (Figure 1).

Surface Topographical Analysis

Test and control implants were both found to have a smooth surface, that is, <0.5 um in average height deviation. There was no statistically significant difference between the two-implant design regarding their height deviation, the density, the ability to retain fluid (the Sci parameter), or the enlarged surface area (Table 2). However, the roughness values for the valleys in between the v-shaped threads of the test implants presented significantly higher values than other parts; however, they were still within the limitations to be regarded as smooth.

ITQ and RTQ

The ITQ value measured by the ITQ device for the control and test groups in the tibia and femur demonstrated no statistical difference. The values were 11.2 Ncm and 17.33 Ncm, respectively, in the tibia, and in the femur 13.33 Ncm and 23.33, respectively. The RTQ values for the control and test groups in tibia were 11.2 Ncm and 17.33 Ncm, respectively, and in the femur, 13.11 Ncm and 23.33 Ncm, respectively (Table 3).

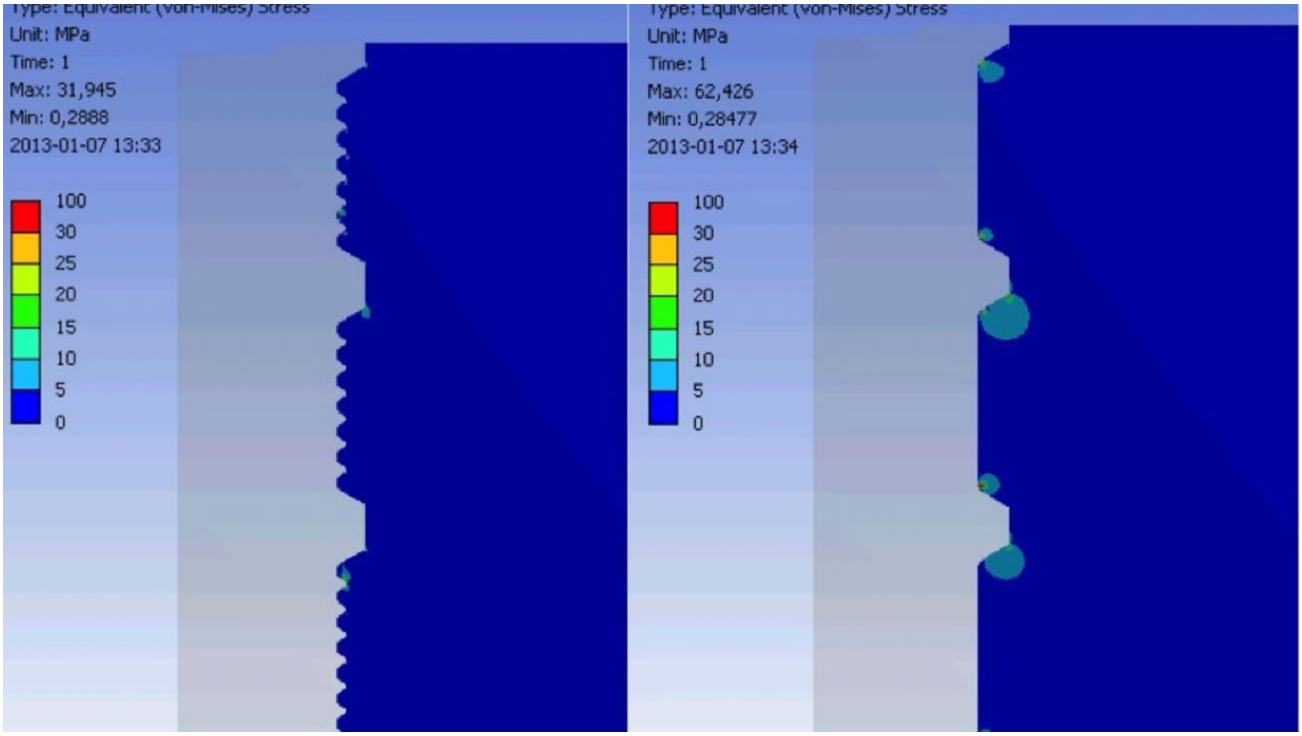

Figure 1 Von Mises stresses generated in test and control implants.

Histomorphometric Analysis

Light microscopy observation revealed that bone formation occured from the periosteal and endosteal surfaces as well as at and around the dislocated bone particles. More primary bone contacts with the implant and bone formation was seen on the surface of the bone trabeculae for the samples in the cancellous bone. Solitary formation of woven bone was also seen in the bone marrow close to blood vessels, but at a distance from existing bone and the implant surface (Figure 2). Osteoblasts were observed toward the underlying layer at the implant surface. It was obvious that implants placed in cancellous bone had more bone at its interface due to bridging between bone trabeculae and the implant surface.

Morphometrical analysis revealed increasing bone contact and bone area values for test implants in the tibia and femur (Tables 4–6) (Figure 3). However, no statistically significant differences were seen for tibia. Femur showed statistical significance in bone volume and bone to implant contact for test implants. New bone formation was observed more often in the micro threads than at the macro threads for test implants (Figure 4). This was statistically verified when pooling the data for the new bone (Table 4).

DISCUSSION

The present study was carried out to analyze the early bone tissue response to micro threads designed along the length of the implant. Previous study from our group has revealed bone affinity to micro threads of such implants in comparison to micro thread less implants.[25] In this study, the effect of micro thread stimulation for bone formation was enhanced with decreasing the macro thread depth, thus increasing the initial contact of micro threads with bone. This initial

TABLE 2 Interferometer Values of Control and Test Implant Surface

Mean ± SD	S_a (um)	S_{ds} (um^{-2})	S_{dr} (%)	Sci
Test	0.25 ± 05	189,422.31 ± 52,397.04	7.59 ± 6.28	1.42 ± 0.32
Control	0.18 ± 0.7	167,721.23 ± 44,382.06	6.34 ± 5.21	1.31 ± 0.21

TABLE 3 Insertion and Removal Torque Analysis in Test and Control Implants

		Tibia (Ncm)		Femur (Ncm)	
ITQ		Test	Control	Test	Control
	Mean	14.22	11.66	19.5	11.4
	SD	14.23	9.82	10.5	9.6
	p Value	.55		.96	
RTQ		Test	Control	Test	Control
	Mean	17.33	11.22	23.33	13.33
	SD	7.5	6.3	6.2	4.7
	p Value	.18		.17	

TABLE 4 Total Bone Volume around the Test and Control Implants

	Tibia		Femur	
	Test	Control	Test	Control
	59.57	56.13	59.57	39.81
	98.05	30.6	70.6	42.45
	61.19	57.63	59.88	34.41
Mean	72.93	48.13	63.35	38.89
SD	21.7	15.2	6.2	4.9
p Value	.10		.10	

contact of micro thread will probably stimulate early bone formation at bone to implant surface.

The stress levels were reduced in a prototype study implant model, when compared with the control implant model, when loaded vertically in the finite element analysis. Addition of micro threads increased the capacity of the implants to take up axial load, by transforming shear stress into compressive stress at the interface.[26–29] Although the values of ITQ and RTQ between test and control were not statistically significant, the mean values in the femur were higher, which may be due to the differences in bone support around the implant in two different types of bone. This has been clearly identified in the histomorphometrical measurements, were seen for femoral with the implants. Although few samples were analyzed the morphometrical measurements showed about placed in the femur possessing approximately two times more of the supporting bone than in the tibia. One reason for this finding may be that the compression and stress distribution was more pronounced in the cancellous bone than in the cortical bone. Higher strains mean higher interface stresses and a higher frictional force, which

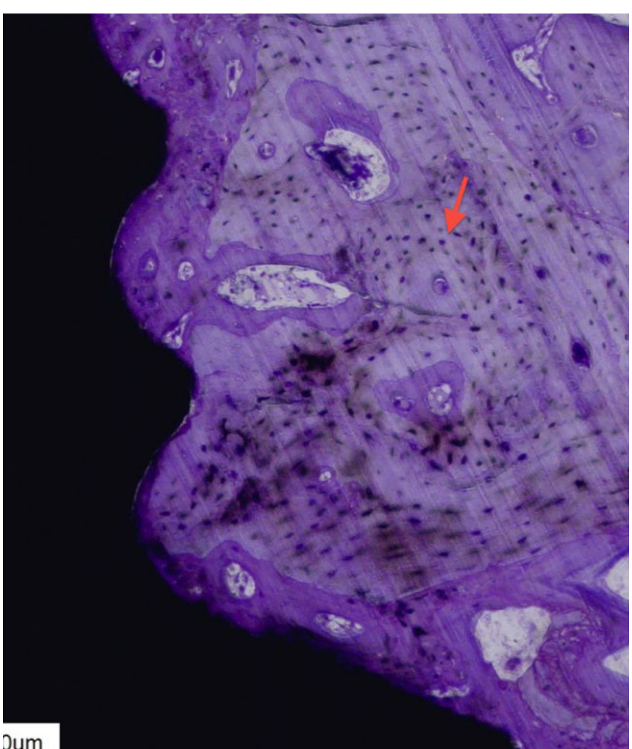

Figure 2 Histological image at ×500 magnification at 4 weeks, of test implant in the femur of the rabbit, showing solitary formation of woven bone in Hervasian system (*red arrow*) and new bone.

TABLE 5 New Bone Volume around Test and Control Implants after 4 Weeks

	Tibia		Femur	
	Test	Control	Test	Control
	19.32	22.74	19.32	1.36
	39.7	13.38	33.68	10.78
	36.01	49.02	18.15	14.11
Mean	47.51	33.63	43.53	23.82
SD	19.07	13.2	22.7	17.2
p Value	.11		.028	

TABLE 6 Bone to Implant Contact around the Test and Control Implants after 4 Weeks

	Test	Control
Mean	42.59	25.84
SD	3.5	11.6
p Value	.04*	

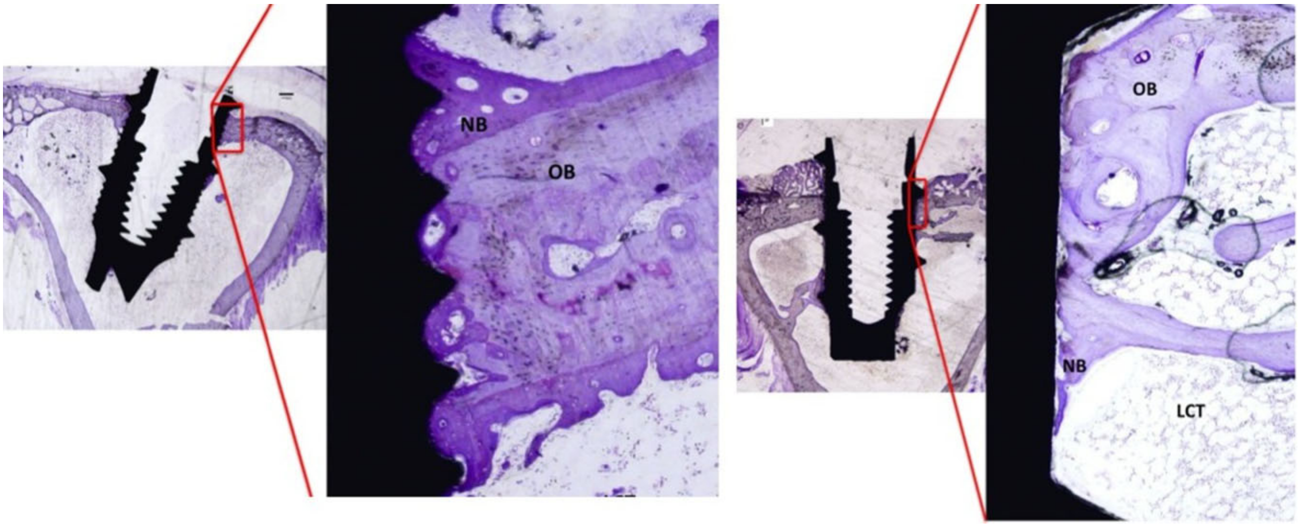

Figure 3 Descriptive light micrographs with the magnified section of the test and control implant in the tibia after 4 weeks of healing. Dense cortical old bone (OB) is surrounded by the new bone (NB). NB is seen along the contour of the micro thread implants. Original magnification 4×. LCT = loose connective tissue.

increases the insertion torque for the test implants.[30] Similarly, higher RTQ values were obtained in the test implants, when compared with control implant group, this probably reflecting the biomechanical properties of the trabecular bone at the femoral site (Table 3).[8,31,32] Histomorphometric analysis values pooled showed, significantly higher amount of bone volume around the test implants in the femoral bone of the rabbit, when compared around the control implants. Wilcoxon test was used as it has large power advantages over *t*-test, when the sample size is small.[33]

The present study found that bone formation occurred comparatively more near the micro threads than on a plane surface, thus indicating that micro threads promote bone stimulation. This was supported by the statistically significant values of bone to implant contact of the study, where micro threads of the test implants were more often in contact in the bone after placement, thus allowing contact guidance, which may allow the cells to migrate in the direction, with the micro threads, as substrates. Thus bone formation would start on the surface and proceeded in the direction toward the surrounding tissues and along the implant surface, as mentioned by Davies and Hosseini and colleagues[34,35] Thus, bone conduction leads to bone formation along the implant surface in parallel with and increased

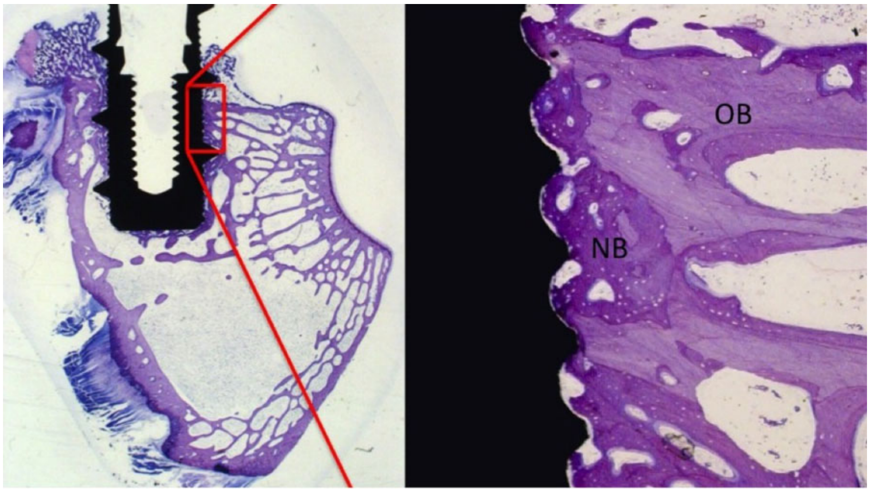

Figure 4 Descriptive light micrographs with the magnified section of the test implant in the femur after 4 weeks of healing. Dense cortical old bone (OB) is surrounded by the new bone (NB). NB is seen along the contour of the micro thread implants. Original magnification 4×.

thickness of the bone by appositional growth. Thus, bone to implant contact was higher for the test implants in the femur bone of the rabbits, suggesting that micro thread configurations offered improved conditions for osseointegration.[31,36]

CONCLUSION

Bone formation occurred more often near the micro threads than on plane surface, thus indicating that micro threads promote bone stimulation. Statistically significant bone growth in femur indicates that cancellous bone is more sensitive to micro thread stimulation.

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