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An animal study of c.p. titanium screws with different surface topographies

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Sixty screw-shaped commercially pure (c.p.) titanium implants were inserted in the tibial and the femoral metaphyses of adult rabbits. The implants were divided into four groups with different surface roughnesses. The surface roughness was characterized before and after implant insertion. One group was left as-machined, this group had an initial R_a value of 0.4 μm. Two groups were blasted with 25 μm sized particles of TiO_2 and Al_2O_3, respectively; corresponding R_a values for these groups were 0.9 μm and 0.8 μm. One group was blasted with 250 μm sized particles of Al_2O_3. The R_a value for this last group was 2.1 μm. After a healing time of 12 weeks the torque necessary for implant removal and histomorphometric evaluations was evaluated. After removal of the implants the R_a values for the four above mentioned groups were 0.9, 1.3, 1.1 and 1.9 μm, respectively. We found a better bone response for implants blasted with 25 μm sized particles compared to an as-machined (turned) surface, but no differences between the implants blasted with 25 μm particles and the implants blasted with 250 μm sized particles.

1. Introduction

A number of studies have investigated different aspects of surface quality for a better understanding of the interplay between living tissue and an implant surface. Investigations have concerned the surface energy, the oxide layer, the surface roughness and the chemical compositions [1–5]. Depending on the implantation site, the demands may differ with respect to surface quality. On the one hand, when penetrating into the oral cavity a rough surface will collect much more bacteria than a smooth one [6–8]. On the other hand several authors have stressed the importance of a rough surface for hard tissue implants [9–12]. Mechanical interlocking may be enhanced by increase of the surface roughness and the stress distribution can be improved [13, 14]. These positive effects of an increased surface roughness must be balanced with the potential negative effects such as a decrease in implant stability [15] and an increase of ion release [16, 17]. The purpose of the present study was to evaluate how well defined surface structures respond to removal torque and to evaluate the bone-to-metal contact for implants with a highly increased surface roughness (blasted with 250 μm particles of Al_2O_3) and for implants with a moderately increased surface roughness (blasted with 25 μm particles of Al_2O_3). Furthermore, we wanted to compare the removal torque for as-machined implants with that of implants blasted with small particles of TiO_2 (25 μm in size). The surface roughness was characterized in a three-dimensional way before and after an implantation time of 12 weeks.

2. Materials and methods

2.1. Animals and anaesthesia

A total of ten adult, New Zealand White rabbits of mixed sexes were used in the present study. They were all aged between 9 and 10 months, with closed physes as evidenced by X-rays. The animals were anaesthetized with intramuscular injections of Hypnorm Vet®, (Janssen Farmaceutica, Belgium) at an initial dose of 0.25 ml per kg body weight and intraperitoneal injections of Apozepam® (Apothekarnes Laboratorium, Norway) at a dose of 0.25 mg per animal. If necessary, additional doses of Hypnorm at a dose of 0.1 ml per kg body weight, were given. Locally to the implant sites, 1.0 ml of 5% Xylocain® (Astra Sweden) was administered in immediate connection to the surgery. Before surgery the animals received an antibiotic, PenoVet® (Boeringer, Denmark) at a dose of 5 ml per animal. An analgesic Temgesic® (Reckitt and Colman, England) was administered immediately after the surgery at a dose of 0.3 ml per rabbit. All ten animals received the latter two drugs 3 days after implantation. Twelve weeks after surgery the animals were euthanized with an overdose of Mebumal Vet®, Nordvacc. Sweden.

2.2. Implant and surface topography characterization

Sixty screw-shaped c.p. (commercially pure) titanium implants with a length of 6 mm, an outer diameter of 3.7 mm and with a pitch height of 0.6 mm were divided into four groups. Group A: 10 screws were left as-machined, i.e. a turned surface. Group B: 10 screws were blasted with 25 μm sized particles of TiO_2. Group C: 20 screws were blasted with 25 μm sized particles of Al_2O_3. Group D: 20 screws were blasted with 250 μm sized particles of Al_2O_3. The blasting process was performed with Basic Due® (Renfert, Germany) blasting equipment. The speed was 3 s/rev and the pressure was 0.36 MPa. The implants were cleaned in an ultrasonic bath (Trichlorethylene and rinsed in absolute ethanol) after the blasting process. A Nikon Measurescope 10® (Nikon, Tokyo, Japan) equipped with a digital counter was used to measure the outer diameter, the core diameter and the pitch height of two randomly chosen screws from each of the four groups. These screws were measured on top, mid and bottom aspects of the screw and the mean value was calculated. For surface topographical characterization an optical profilometer developed for three-dimensional measurements, TopScan 3D® (Heidelberg Instruments GmbH, Germany) was used. A He–Ne laser spot of about 1 μm in diameter is scanned over the surface. High resolution is achieved due to the confocal design of the profilometer. Numerical values for different surface roughness parameters and visual images are produced by appropriate software. The system has been described in more detail previously [18]. Two screws, randomly chosen, were measured on three flanks, one thread-top and one thread-bottom each. The measuring area was 240 × 245 μm for all measurements. A Gaussian filter was used to exclude form and waviness from roughness. The filter size was set to 30 × 30 μm. For numerical descriptions we used seven different roughness parameters, five were pure height descriptive and two include information from space, Δ_q and λ_q.

R_a is the arithmetic mean of the departures of the roughness profile from the mean line, measured in micrometres. This parameter gives a good general description of height variations but is insensitive to wavelength and occational high peaks and low valleys.

R_q is the root mean square parameter corresponding to R_a, measured in micrometres.

R_t is the maximum peak to valley height in the evaluation area, measured in micrometres. This parameter is an extreme value and may have large scatter due to random sampling.

R_{sk} or skewness is the measure of the symmetry of the profile, a surface with more valleys than peaks has a negative skewness value, and a positive skewness value means there are more peaks than valleys.

R_{ku} is the measure of the sharpness of the surface profile, a perfect Gaussian distribution has a kurtosis value of 3, if the area has relatively more peaks and valleys the kurtosis value will be > 3.

Δ_q is the root mean square slope of the profile throughout the assessment area, measured in degrees.

λ_q is the root mean square measure of the spacings between local peaks and valleys, taking into account their relative amplitudes and individual spatial frequencies, measured in micrometres. After the measuring procedure all implants were sterilized in an autoclave.

2.3. Surgical technique and implant insertion

Each animal received six implants: in every left femur one turned screw and in every right femur one screw blasted with 25 μm sized TiO_2 particles; in the left proximal methaphysis of the tibia two screws blasted with 250 μm sized particles of Al_2O_3 and in the right tibial methaphysis two screws blasted with 25 μm sized Al_2O_3 particles; the left leg of the rabbit served as a control and the right leg served as the test sample. The tibial implants were placed 5 mm apart. A controlled surgical technique was used including careful drilling with low rotatory speed and profuse saline cooling. Immediately after surgery all rabbits were allowed full weight bearing.

2.4. Torque measurements, histomorphometric measurements and preparation of specimens

The torque necessary to unscrew the implants implanted in the femur ($n = 20$) and the implants placed most distally in the left and in the right tibia ($n = 20$) was measured with a Tohnichi 6 BTG-N® (Tohnici MFG, Japan) with a measuring range from 0–74 N cm. The tibial implants placed most proximally ($n = 20$) were prepared for histomorphometrical evaluation. These implants with surrounding bone were fixed in 4% neutral buffered formaldehyde and embedded in light-curing resin, Technovit 7200 VLC (Kultzer & Co., Germany). Cutting and grinding were performed in an Exakt sawing machine and grinding equipment as described by Donath [19]. The approximate 10 μm thick sections were stained with toluidine blue. The histomorphometric analysis was performed with help of a Leitz Microvid equipment connected to a PC computor. All measurements were calculated with a 10 × magnification objective. Measurements of percentage bone-to-metal contact in all threads around the implant as well as the three best consecutive threads in the cortical region (representing the major part of the cortical bone) were calculated. The amount of bone in the thread area was examined in a similar manner. Comparison was made only between control and test side in the same animal.

2.5. Surface topography characterization after 12 weeks in rabbit bone

One screw from each group was randomly chosen after the torque measurement. These four screws were placed in test tubes containing EDTA (ethylene diamine tetra acetic acid) solution and kept at room temperature for 8 weeks in order to dissolve hard tissue remnants on the implant surface; afterwards they were ultrasonically cleaned in 70% ethanol. Each screw was measured on top, mid and bottom aspects of the screw on nine sites each, three flanks, three tops and three valleys. The measurements were the same as those done before surgery.

Wilcoxon signed rank test was used to calculate p-values for torque measurements and for the histomorphometric analysis. Each animal served as its own control.

3. Results

With respect to the surface analysis, we found the screws in group A, (turned surface) to be the smoothest ones included in this study. (Fig. 1a, 1b) They had an anisotropic surface, i.e. the surface texture has a clear direction; this is in contrast to the screws of group B and C where the screws had an isotropic structure, i.e. there is no predominant direction of the surface texture. Group B and C blasted with the same size (25 μm) blasting particles although of different materials (TiO_2 and Al_2O_3, respectively) exhibited a very similar surface structure (Figs 2a, 2b and 3a, 3b). Group D screws blasted with 250 μm sized particles of Al_2O_3 demonstrated the roughest surface evaluated in the present study. The surface structure in group D was inhomogenous, small rather smooth areas were found between areas with high peaks and deep valleys (Fig. 4a, 4b) (Table I). The surface analysis performed on the implants after an insertion time of 12 weeks in the rabbit bone demonstrated some changes compared to the surface analysis performed before surgery. All four groups had a more negative value of skewness parameter. Group A, B and C had all increased their surface roughness, group A more than group B and C (Fig. 5). Group D had slightly decreased surface roughness (Fig. 6). The R_t value for

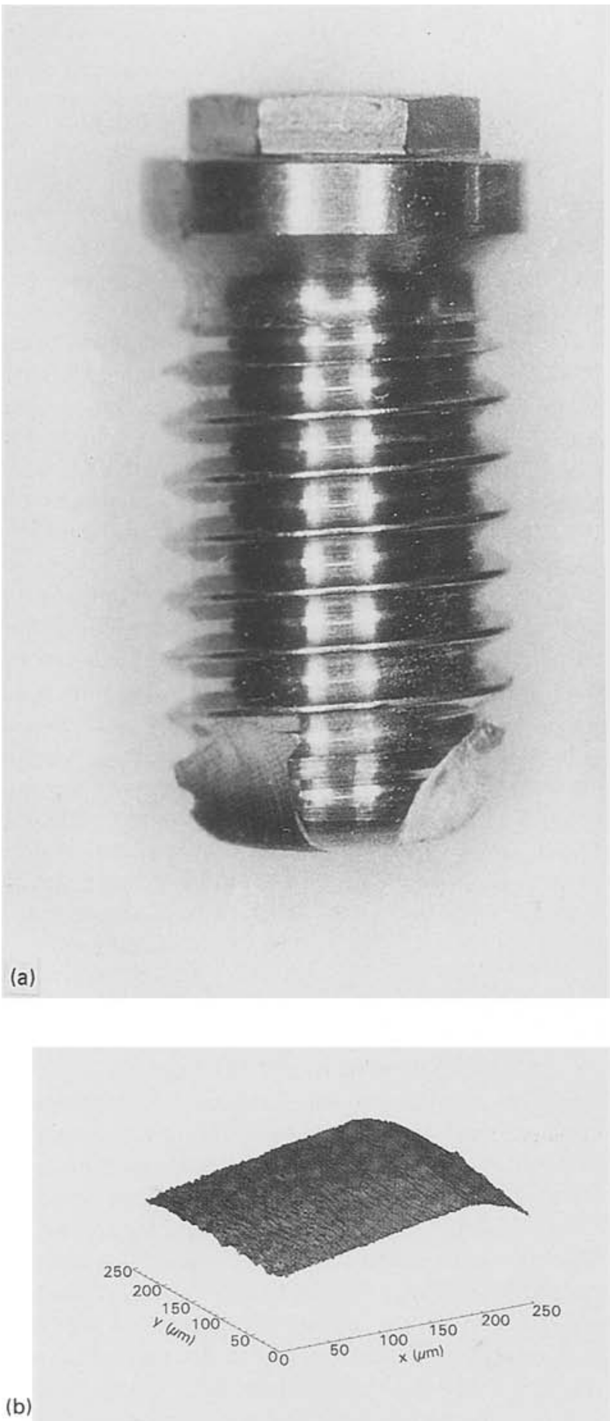

Figure 1 (a) A c.p. titanium screw (turned) 6 mm in length, diameter of 3.7 mm and pitch height 0.6 mm. (b) A topographical image of a thread flank of a turned c.p. titanium screw. Thread flank.

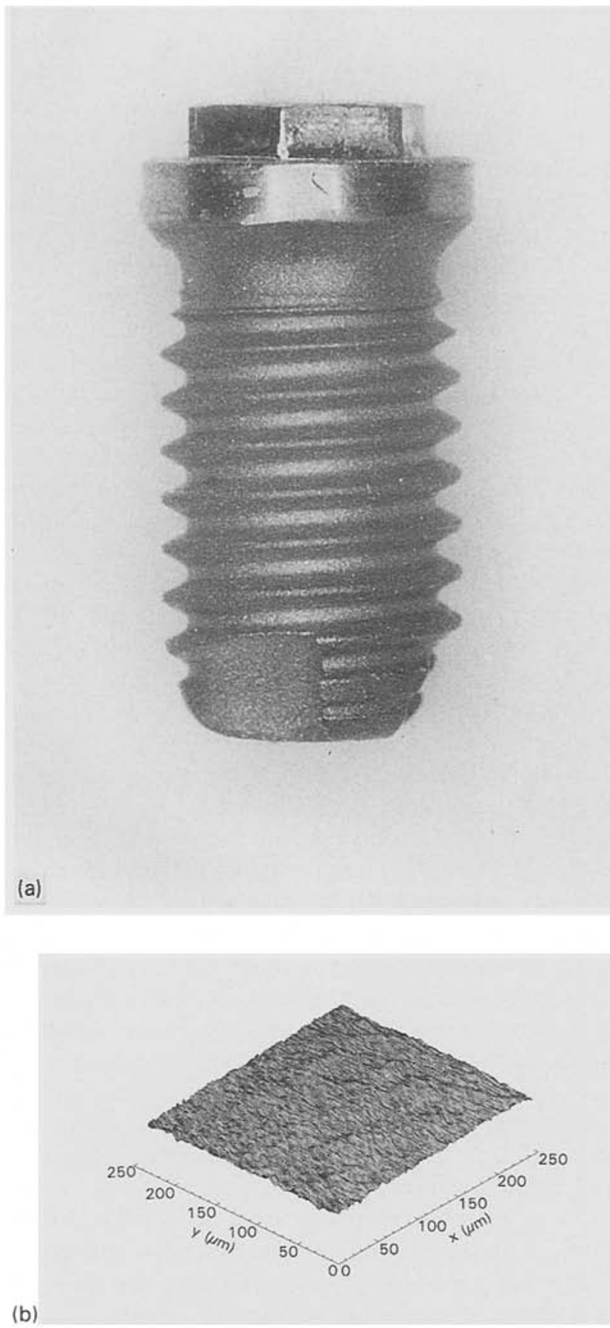

Figure 2 (a) A c.p. titanium screw 6 mm in length, diameter 3.7 mm, pitch height 0.6 mm. Surface blasted with 25 μm sized TiO_2 particles. (b) Surface structure of a titanium oxide blasted (25 μm particles) c.p. titanium screw. Thread flank.

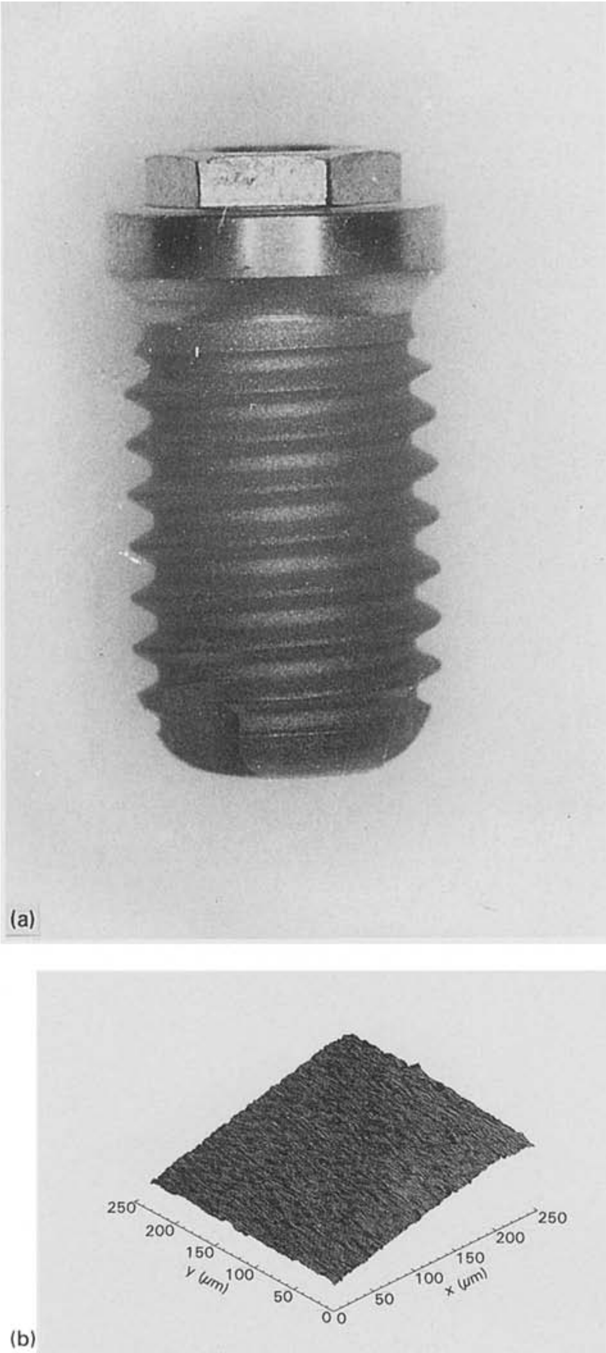

Figure 3 (a) A c.p. titanium screw 6 mm in length, diameter 3.7 mm, pitch height 0.6 mm. Surface blasted with 25 μm sized Al_2O_3 particles. (b) Surface structure of an aluminium-oxide blasted (25 μm particles) c.p. titanium screw. Thread flank.

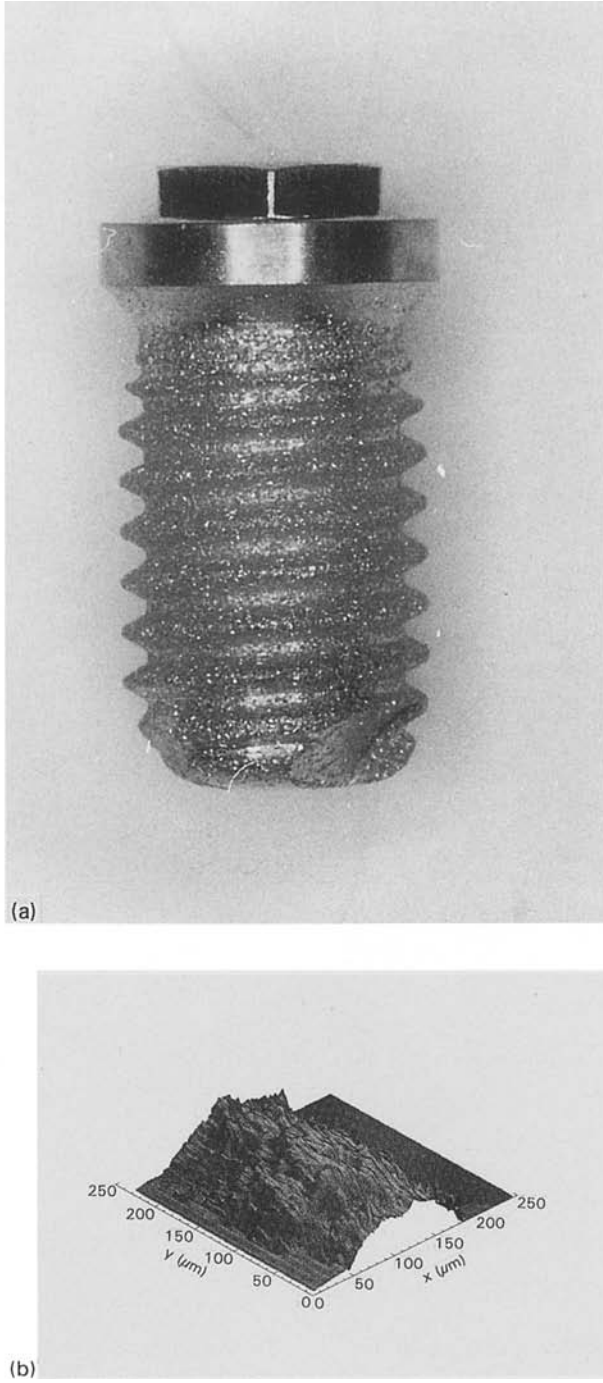

Figure 4 (a) A c.p. titanium screw 6 mm in length, diameter 3.7 mm, pitch height 0.6 mm. Surface blasted with 250 μm sized Al_2O_3 particles. (b) Rough surface demonstrated by a screw blasted with 250 μm Al_2O_3 particles.

group D was about half that before implant insertion, while the R_t value had increased for group A and remained similar for groups B and C. In general, the height descriptive parameters had been more influenced by insertion and removal of the implants than had the space and height descriptive parameter (Δ_q and λ_q) (Table II).

We used the Nikon Measurescope 10 equipment to ensure that the abrasive blasting had not severely diminished the screw outer as well as core diameter. Furthermore, we measured the pitch height. We found very small differences between the four different groups of implants. The small changes for the three groups treated with different blasting procedures were within the variation that could occur among the unblasted (as-machined) implants (Table III).

The torque necessary to loosen the screws was significantly higher for the implants blasted with 25 μm sized particles of TiO_2 and inserted in the right femur (group B) than that for the turned implants inoperated in the left femur (group A). The mean value of the torque to unscrew the implants in the right femur was 45.4 N cm whereas the mean value in the left femur was 38.4 N cm ($p = 0.010$). There was not a statistically significant difference between the implants in the right tibia (group C) and the implants in the left tibia (Group D). The mean value for the screws in the right

TABLE I The mean value of seven commonly used surface roughness parameters characterizing the surface structure for differently treated titanium implants

	R_a (μm)	R_q (μm)	R_t (μm)	R_{sk}	R_{ku}	Δ_q(°)	λ_q (μm)
Group A							
Turned surface							
Screw 1	0.42	0.59	9.93	− 0.08	19.09	23.57	8.86
Screw 2	0.34	0.49	6.31	0.17	9.84	20.23	8.62
Mean of 1 + 2	0.38	0.54	8.23	0.04	14.47	21.90	8.74
SD 1 + 2	0.2	0.2	3.8	1.9	9.6	6.9	1.1
Group B							
Blasted surface							
TiO_2 25 μm							
Screw 1	0.85	1.18	18.23	0.37	8.90	35.71	11.77
Screw 2	0.91	1.04	20.2	0.57	10.76	37.46	12.17
Mean of 1 + 2	0.88	1.11	19.23	0.47	9.83	36.58	11.97
SD	0.2	0.3	5.4	1.0	6.4	5.6	1.1
Group C							
Blasted surface							
Al_2O_3 25 μm							
Screw 1	0.84	1.29	20.7	1.43	17.89	35.86	12.87
Screw 2	0.80	1.12	16.46	0.42	10.11	36.62	11.09
Mean of 1 + 2	0.82	1.21	18.57	0.93	14.0	36.24	11.98
SD	0.2	0.3	5.6	1.1	9.4	3.5	2.4
Group D							
Blasted surface							
Al_2O_3 250 μm							
Screw 1	2.11	3.04	41.18	0.12	8.11	55.27	19.72
Screw 2	2.11	3.01	45.00	− 0.14	8.48	56.12	19.33
Mean of 1 + 2	2.11	3.02	43.09	− 0.01	8.29	55.69	19.53
SD	0.1	0.3	6.9	0.4	2.6	2.2	1.5

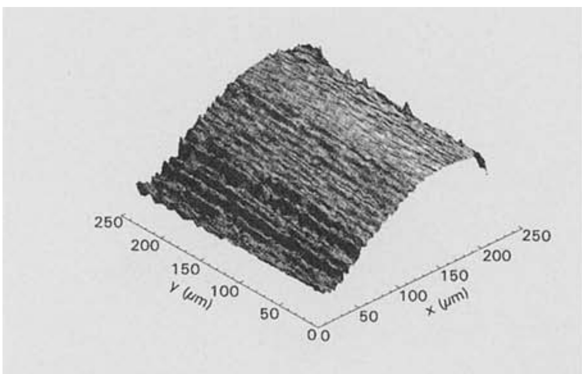

Figure 5 Topographical image of a turned c.p. titanium screw after an insertion time of 12 weeks. An increased surface roughness is shown compared to before implant insertion, demonstrated in Fig. 1b.

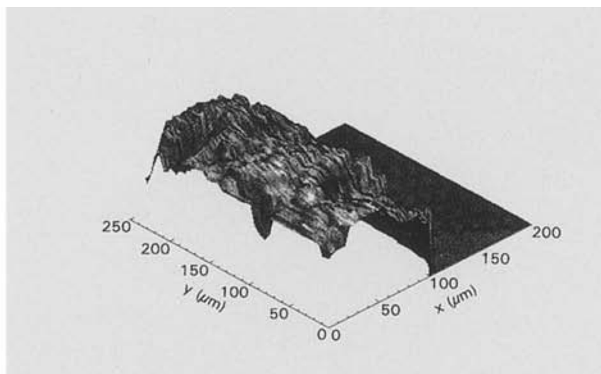

Figure 6 Thread top of a c.p. titanium screw blasted with 250 μm particles of Al_2O_3 after implantation in the rabbit bone. The peaks have decreased in size and the pits in the surface are now more visible compared to Fig. 4b.

tibia was 41.7 N cm whereas the mean value for the left tibia was 39.8 N cm ($p = 0.238$) (Table IV).

The histomorphometric analysis demonstrated more bone-to-metal contact when calculated over the three best consecutive threads for the implants blasted with 25 μm Al_2O_3 (mean value 46.4% compared to 39.2% for the implants blasted with 250 μm Al_2O_3 particles) ($p = 0.030$). When calculating over all threads in the screw, there was no statistically significant difference between the left and the right side. Mean value for the right tibial implants (25 μm, group C) was 27.6% and mean value for the left tibial implants (250 μm, group D) was 24.3% ($p = 0.439$) (Table V) The percentage amount of bone in the thread region was similar for right and left side. Calculating over the three best consecutive threads in the screws inserted in the right side (25 μm, group C) the amount of bone was 72.6%, compared to the screws inserted in the left tibia (250 μm, group D) where the amount of bone was 68.7% ($p = 0.166$). Calculating over all threads in the screw implanted in the right side the amount of bone was 36.9% and the corresponding value for the left side was 34.4% ($p = 0.399$) (Table VI) (Figs 7 and 8)

4. Discussion

In the present study we found that an increase in blasting particle diameter resulted in an increased surface roughness, but the type of blasting material (TiO_2 or Al_2O_3) did not seem to influence the surface

TABLE II The mean value of seven surface roughness parameters after 12 weeks in rabbit bone. One screw, randomly chosen from each group was measured on nine sites

	R_a (μm)	R_q (μm)	R_t (μm)	R_{sk}	R_{ku}	Δ_q(°)	λ_q (μm)
Group A							
Turned surface							
Mean of 9 measurements	0.88	1.16	15.32	− 0.46	8.00	41.24	10.94
SD	0.2	0.4	4.6	0.7	2.4	7.1	1.4
Group B							
Blasted surface							
TiO_2 25 μm							
Mean of 9 measurements	1.34	1.77	21.46	− 0.35	6.79	46.69	13.77
SD	0.3	0.3	6.9	0.6	4.9	4.7	1.4
Group C							
Blasted surface							
Al_2O_3 25 μm							
Mean of 9 measurements	1.06	1.43	14.55	− 0.50	5.4	39.8	12.9
SD	0.2	0.3	4.8	0.6	1.5	6.8	1.2
Group D							
Blasted surface							
Al_2O_3 250 μm							
Mean of 9 measurements	1.93	2.63	27.55	− 0.33	5.67	47.1	20.04
SD	0.5	0.7	11.1	0.5	2.0	9.9	3.8

TABLE III Measurements of the outer diameter, core diameter and pitch height of the four groups after surface modification

	Outer diameter (mm)	Core diameter (mm)	Pitch height (mm)
Group A			
Turned surface			
Screw 1	3.693	3.071	0.598
Screw 2	3.701	3.075	0.596
Mean screw 1 + 2	3.697	3.073	0.597
SD 1 + 2	0.008	0.005	0.002
Group B			
Blasted surface			
TiO_2 25 μm			
Screw 1	3.697	3.084	0.598
Screw 2	3.693	3.076	0.599
Mean screw 1 + 2	3.695	3.079	0.598
SD 1 + 2	0.008	0.005	0.006
Group C			
Blasted surface			
Al_2O_3 25 μm			
Screw 1	3.660	3.053	0.603
Screw 2	3.708	3.104	0.598
Mean screw 1 + 2	3.684	3.078	0.600
SD 1 + 2	0.029	0.029	0.006
Group D			
Blasted surface			
Al_2O_3 250 μm			
Screw 1	3.704	3.083	0.595
Screw 2	3.699	3.088	0.595
Mean screw 1 + 2	3.702	3.085	0.595
SD 1 + 2	0.004	0.005	0.012

structure or the tissue reactions [20]. We also found the blasting procedure to preserve the major shape of the implant. The observed differences in bone response, therefore, can be referred to the different surface structures that were investigated. The femoral implants demonstrated significantly higher removal torques for implants blasted with 25 μm particles of TiO_2 compared to the as-machined, turned implants. This is consistent with a previous study [21], where we found when comparing implants inserted in the tibial methaphysis, that higher removal torques were required for the TiO_2 blasted specimens compared to the as-machined ones. This may indicate that a slight increase of surface roughness compared to as-machined implants is preferable for bone fixation in cancellous as well as compact bone, at least over a short follow-up time.

TABLE IV Removal torques of tibial and femoral implants as machined and blasted with 25 μm and 250 μm sized particles, 12 weeks after insertion

Rabbit number	Removal torque (N cm)			
	Right femur Blasted (TiO_2 25 μm)	Left femur Turned	Right tibia Blasted (Al_2O_3 25 μm)	Left tibia Blasted (Al_2O_3 250 μm)
1	51	38	36	22
2	63	56	41	48
3	36	37	36	44
4	35	38	31	38
5	35	21	44	35
6	38	25	27	28
7	51	50	51	50
8	37	31	41	41
9	42	33	55	41
10	66	55	55	67
Mean value	45.4	38.4	41.7	39.8
SD	11.7	11.9	9.67	13.5
Min–Max value	35–66	21–56	27–55	22–67

p-value calculated with Wilcoxon signed rank test
Femur p-value = 0.01
Tibia p-value = 0.238

TABLE V Measured percentage bone-to metal contact of c.p. titanium implants blasted with 25 and 250 μm sized aluminum-oxide particles 12 weeks after insertion. Standard deviation and minimum-maximum values within parenthesis

Thread number	Bone–metal contact (%)	
	Right tibia Al_2O_3 25 μm	Left tibia Al_2O_3 250 μm
1	37.5	17.5
2	42.9	35.4
3	44.6	45.4
4	30.7	31.6
5	18.6	24.8
6	11.8	21.7
7	6.2	17.8
All threads around the screw	27.6 (11) (11–46)	24.3 (5.9) (25–47)
Three consecutive best threads	46.4 (13) (25–66)	39.2 (5.9) (25–47)

p-value calculated with Wilcoxon signed rank test.
p-value all threads in the tibia $p = 0.439$
p-value three consecutive best threads in the tibia $p = 0.030$

TABLE VI The average amount of bone (percentage) of thread area of c.p. titanium implants blasted with two sizes of aluminum oxide particles, 12 weeks after insertion in the tibia. Standard deviation and minimum–maximum values within parenthesis

Thread number	Bone–in thread area (%)	
	Right tibia Al_2O_3 25 μm	Left tibia Al_2O_3 250 μm
1	68.5	51.4
2	68.8	79.6
3	64.4	63.3
4	32.5	31.3
5	14.2	6.0
6	7.7	7.4
7	5.8	9.3
All threads around the screw	36.9 (8.3) (25–52)	34.9 (6.0) (24–44)
Three consecutive best threads	72.6 (8.2) (61–86)	68.8 (10.3) (48–82)

p-value calculated with Wilcoxon signed rank test.
p-value all threads in the tibia $p = 0.399$
p-value three consecutive best threads in the tibia $p = 0.166$

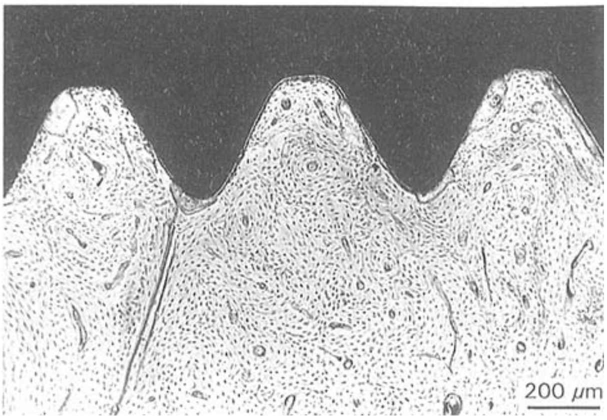

Figure 7 Ground section of a c.p. titanium screw blasted with 25 μm particles of Al_2O_3.

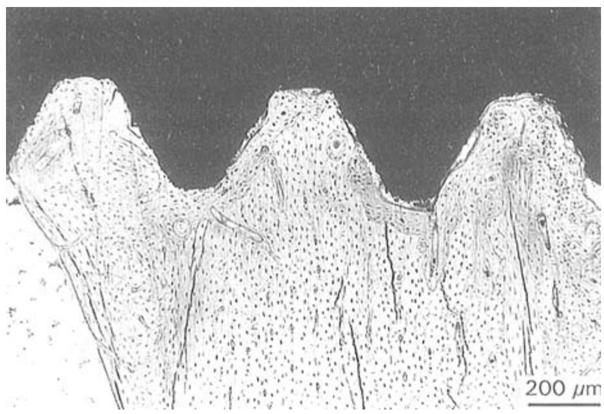

Figure 8 Ground section of a c.p. titanium screw blasted with 250 μm Al_2O_3 particles. The percentage of bone in contact with the metal surface is somewhat less compared to that shown in Fig. 7.

This study demonstrates no differences between the two groups of blasted implants inserted in the tibia. Buser *et al.* [9], concluded that "the extent of bone–implant interface is positively correlated with an increasing roughness of the implant surface". However, the results from the present study indicate no advantage (for a short follow-up time) to increasing the blasting particles from 25 μm to 250 μm and thereby increasing the surface roughness from a R_a value of 0.8 μm to a R_a value of 2.1 μm. In further studies we will attempt to add knowledge about bone reactions to various surface roughness at a longer follow-up time of 1 year.

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References

1. C. APARICIO and J. OLIVÉ, *Int. J. Oral Maxillofac. Implants* **7** (1992) 94.
2. A. CIGADA, M. CABRINI and P. PEDEFERRI. *J. Mater. Sci. Mater. Med.* **3** (1992) 408.
3. C. KLAUBER, L. J. LENZ and P. J. HENRY. *Int. J. Oral Maxillofac. Implants* **5** (1990) 264.
4. D. W. MURRAY, T. RAE and N. RUSHTON. *J. Bone Joint Surg.* **71** (1989) 632.
5. E. VARGAS, R. E. BAIER and A. E. MEYER. *Int. J. Oral Maxillofac. Implants* **7** (1992) 338.
6. J. McCOLLUM, R. B. O'NEAL, W. A. BRENNAN, T. E. VAN DYKE and J. A. HORNER. *J. Peridontol.* **63** (1992) 802.
7. M. QUIRYNEN, M. MARECHAL, H. J. BUSSCHER, A. H. WEERKAMP and D. van STEENBERGHE. *J. Clin. Periodontal.* **17** (1990) 138.
8. M. QUIRYNEN, H. C. VAN DER MEI, C. M. L. BOLLEN, A. SCHOTTE, M. MARECHAL, G. I. DOORNBUSCH, I. NAERT, H. J. BUSSCHER and D. VAN STEENBERGHE. *J. Dent. Res.* **72** (1993) 1304.
9. D. BUSER, R. K. SCHENK, S STEINEMANN, J. P. FIORELLINI, C. H. FOX and H. STICH. *J. Biomed. Mater. Res.* **25** (1991) 889.
10. L. CARLSSON, T. RÖSTLUND, B. ALBREKTSSON and T. ALBREKTSSON. *Int. J. Oral Maxillofac. Implants* **3** (1988) 21.
11. J. L. RICCI, J. M. SPIVAK, N. C. BLUMENTHAL and H. ALEXANDER. in "The bone–biomaterial interface, edited by J. E. Davis (University of Toronto Press, 1991) p. 334.
12. D. M. BRUNNET; B. CHEHROUDI and T. R. L. GOULD. in Proceedings of the Second International Congress on Tissue Integration in Oral, Orthopedic Maxillofacial Reconstruction, Rochester, Minnesota, September 1990, edited by W. R. Laney and D. E. Tolman (Quitessence Books, Chicago, 1992) p. 21.
13. S. G. STEINEMANN, J. EULENBERGER, P-A. MAEUSLI and A. SCHROEDER. in "Biological and biomechanical performance of biomaterials, edited by P. Christel, A. Meunier and A. J. C. Lee, (Elsevier Science Publishers B. V., Amsterdam, 1986) p. 409.
14. A. YAMAGAMI, Y. NISHIO and Y. YOSHIHARA, in Fourth World Biomaterials Congress, Berlin April 1992, p. 386.
15. L. CARLSSON, T. RÖSTLUND, B. ALBREKTSSON and T. ALBREKTSSON. *Acta. Orthop. Scand.* **59** (1988) 272.
16. J. F. OSBORN, P. WILLICH and N. MEENEN. in "Clinical implant materials" (Elseviers Science Publishers, Amsterdam, 1990) p. 75.
17. P. DUCHEYNE, G. WILLEMS, M. MARTENS and J. HELSEN. *J. Biomed. Mater. Res.* **18** (1984) 293.
18. A. WENNERBERG, T. ALBREKTSSON, H. ULRICH and J. J. KROL. *J. Biomed. Engng.* **14** (1992) 412.
19. K. DONATH. *Der Präparator* **34** (1988) 197.
20. A. WENNERBERG, T. ALBREKTSSON, C. JOHANSSON and B. ANDERSSON, *Biomaterials* (submitted).
21. A. WENNERBERG, T. ALBREKTSSON, B. ANDERSSON and J. J. KROL, *Clin. Oral Impl. Res.* (accepted).

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