Anchorage of Titanium Implants with Different Surface Characteristics: An Experimental Study in Rabbits

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ABSTRACT

Purpose: To compare the anchorage of titanium implants with different surface roughness and topography and to examine histologically the peri-implant bone after implant removal.

Materials and Methods: Screw implants with five different surface topographies were examined: (1) turned ("machined"), (2) TiO_2-blasted with particles of grain size 10 to 53 µm; (3) TiO_2-blasted, grain size 63 to 90 µm; (4) TiO_2-blasted, grain size 90 to 125 µm; (5) titanium plasma-sprayed (TPS). The surface topography was determined by the use of an optical instrument. Twelve rabbits, divided into two groups, had a total of 120 implants inserted in the tibiae. One implant from each of the five surface categories was placed within the left tibia of each rabbit. By a second operation, implants were installed in the right tibia, after 2 weeks in group A and after 3 weeks in group B. Fluorochrome labeling was performed after 1 and 3 weeks. Removal torque (RMT) tests of the implants were performed 4 weeks after the second surgery in group A and 9 weeks after the second surgery in group B. Thus, in group A, two healing groups were created, representing 4 and 6 weeks, respectively. The corresponding healing groups in group B were 9 and 12 weeks. The tibiae were removed, and each implant site was dissected, fixed, and embedded in light-curing resin. Ground sections were made, and the peri-implant bone was analyzed using fluorescence and light microscopy.

Results: The turned implants had the lowest S_a and S_y values, whereas the highest scores were recorded for the TPS implants. The corresponding S_a and S_y values for the TiO_2-blasted implants were higher when a larger size of grain particles had been used for blasting. At all four observation intervals, the TPS implants had the highest and the turned implants the lowest RMT scores. The differences between the various TiO_2-blasted implants were, in general, small, but the screws with the largest S_a value had higher RMT scores at 6, 9, and 12 weeks than implants with lower S_a values.

The histologic analysis of the sections representing 6, 9, and 12 weeks revealed that fractures or ruptures were present in the marginal, cortical peri-implant bone. In such sections representing the TPS and TiO_2-blasted implant categories, ruptures were frequently found in the zone between the old bone and the newly formed bone, as well as within the newly formed bone.

Conclusions: The present study demonstrated that a clear relation exists between surface roughness, described in S_a values, and implant anchorage assessed by RMT measurements. The anchorage appeared to increase with the maturation of bone tissue during healing.

KEY WORDS: anchorage, implant, integration, surface characteristics, surface roughness, titanium

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.[1–3] Although, currently, the specific surface characteristics for an optimal bone integration are not known, findings from in vitro studies indicate that a certain degree of surface roughness may have a positive effect on adsorption of molecules, local factor production,

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and proliferation and differentiation of cells at the implant surface.[4]

Differences in topography and roughness can be obtained by treating the titanium surface with additive and reductive techniques. Titanium-plasma spraying (TPS) is an example of an additive technique: titanium particles are coated onto the implant surface at a high temperature.[5] Titanium dioxide blasting represents a reductive technique, in which a plastic deformation of the surface is obtained by blasting particles of TiO_2 toward the surface.[6] This technique was introduced to increase the roughness without adding foreign elements to the surface and to enable the surface configuration to be changed by using different particle sizes for the blasting procedure.[6]

Furthermore, in vivo studies have demonstrated a higher degree of bone integration of implants with a rough surface compared to implants with a comparatively smoother surface.[7–10] Although the studies referred to included histologic and biomechanical tests of implants with different surface characteristics, apparently there was histologic evaluation of the peri-implant bone at the implants used for the biomechanical tests.

The aim of the present study was to compare the anchorage of titanium implants with different surface roughness and topography and to examine histologically the peri-implant bone bed adjacent to the removed implants.

MATERIALS AND METHODS

A total of 130 screw implants of commercially pure titanium (length 6 mm, outer diameter 3.5 mm) were used in the present experiment (Figure 1). The implants were divided into the following five surface categories:

1. Turned (machined) surface
2. TiO_2-blasted with particles of grain size 10 to 53 µm
3. TiO_2-blasted, grain size 63 to 90 µm
4. TiO_2-blasted, grain size 90 to 125 µm
5. titanium plasma-sprayed (TPS) with titanium powder of grain size 50 to 100 µm, coated to the surface with an argon gas stream (temperature approx. 15,000°C) to about 600 m/s during the coating process.

The blasting procedure was performed at 4 bar overpressure in 10 seconds per implant.

In vitro examinations of the titanium surface were performed at two implants selected at random from each group. The 10 implants were measured in six areas of 30 µm × 30 µm each; two measurements were made at a top position, two at a midposition, and two at a bottom position of the implants. An optical laser roughness tester instrument (UBM Microfocus 1080 D7505 Ettlinger, Messtechnich, GMBH, Germany) was used for the profilometric examination. The instrument was equipped with an optical sensor using a laser with a wave length of 780 nm and a spot diameter of 1 µm. Five surface parameters, S_a, S_y, S_{ku}, S_{sk}, S_{dr}, were selected for the numerical characterization (Table 1).

S_a = arithmetic mean of deviation of the roughness surface from the mean line.
S_y = maximum peak-to-valley height
S_{ku} = kurtosis of height distribution
S_{sk} = skew of height distribution
S_{dr} = developed surface area ratio.

The selected parameters have been described in detail by Wennerberg.[11] The values obtained for all six areas of each implant were collected, and mean values and variances were calculated for each surface category. In addition, the five different titanium-treated surfaces were examined in a Philips 50 scanning electron microscope (SEM). A representative thread for each group of implants is illustrated in Figure 2.

Animals and Experimental Design

A total of 120 implants, 24 from each surface group, were inserted in 12 female white rabbits (*Oryctolagus cuniculus*), between 9 and 12 months of age and with a weight of 3.4 to 4.0 kg. Housing and feeding of the ani-

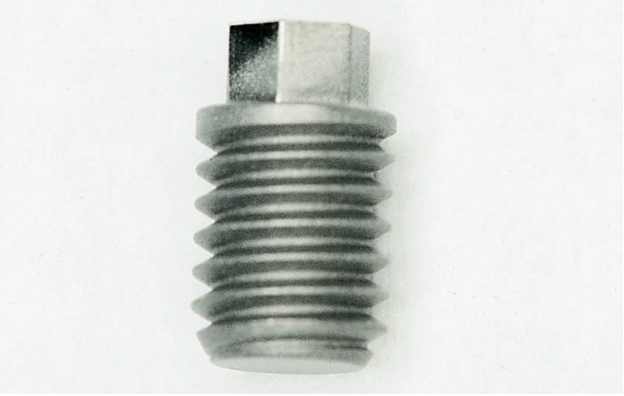

Figure 1. One of the titanium screw implants blasted with 63–90 µm particles of TiO_2. Length 6 mm, diameter 3.5 mm.

TABLE 1. Surface Roughness of Experimental Titanium Implant Surfaces

		Surface Parameter (Mean ± SD%)				
Group	Surface Treatment	S_a (μm)	S_y (μm)	S_{sk}	S_{ku}	S_{dr}
1	Turned (machined)	0.37 ± 0.7%	3.86 ± 3.2%	0.70 ± 10.5%	4.48 ± 7.1%	1.37 ± 1.0%
	TiO_2-blasted					
2	10–53 μm*	1.05 ± 4.1%	9.33 ± 3.3%	–0.55 ± 9.7%	3.58 ± 3.7%	2.76 ± 1.8%
3	63–90 μm*	1.16 ± 0.6%	11.34 ± 2.3%	–0.63 ± 4.9%	4.27 ± 3.6%	2.95 ± 1.7%
4	90–125 μm*	1.45 ± 1.9%	14.34 ± 2.3%	–1.37 ± 2.6%	6.77 ± 1.0%	3.05 ± 1.6%
5	Titanium plasma-sprayed	3.54 ± 1.8%	32.41 ± 4.1%	0.48 ± 25.8%	4.09 ± 6.3%	5.18 ± 0.7%

S_a = average height deviation from mean plane; S_y = maximum peak-to-valley height; S_{sk} = skew of height distribution; S_{ku} = kurtosis of height distribution; S_{dr} = developed surface area ratio.
*Grain size.

mals were according to national standards for laboratory animals. The study protocol was approved by the National Ethical Committee on Animal Experiments.

Anesthesia was performed by intramuscular injection of a combination of fentanyl and fluanison (Hypnorm® Vet., Janssen Pharmaceutica, Brussels, Belgium)

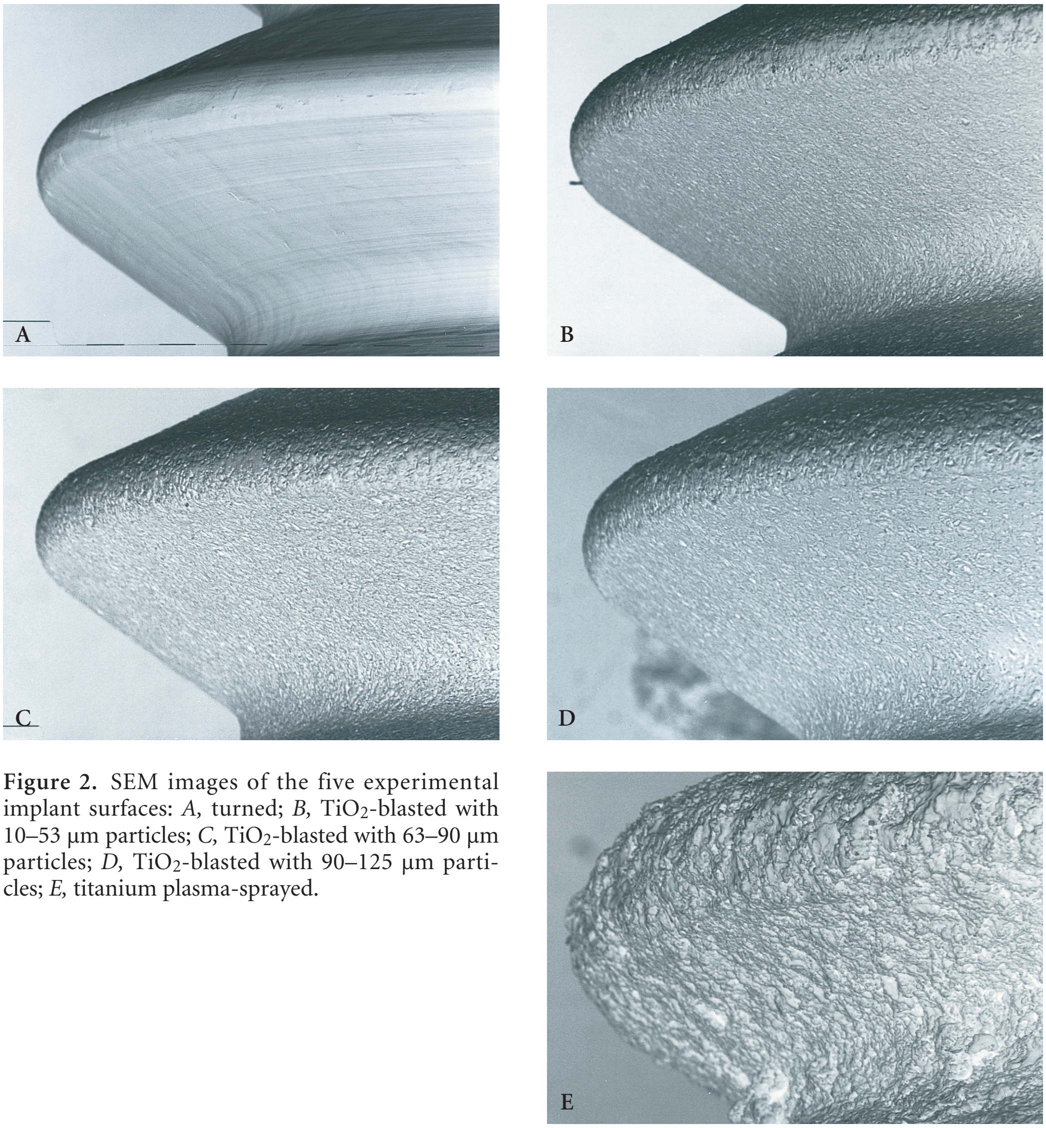

Figure 2. SEM images of the five experimental implant surfaces: *A,* turned; *B,* TiO_2-blasted with 10–53 μm particles; *C,* TiO_2-blasted with 63–90 μm particles; *D,* TiO_2-blasted with 90–125 μm particles; *E,* titanium plasma-sprayed.

at a dose of 0.8 mL/kg body weight, following premedication with intraperitoneal injections of diazepam (Valium, Roche, France) at a dose of 3.0 mg per animal. Postoperatively the animals received benzylpenicillin 200,000 IE (Streptocillin vet, Novo, Copenhagen, Denmark) at a dose of 0.12 mL/kg body weight.

The 12 rabbits were divided into two main groups: group A and group B. In both groups, an incision was made at the medial side of the proximal left tibia. After the skin and fascia were reflected, five unicortical bone preparations, about 7 mm apart, were made. A series of twist drills with diameters from 2.5 mm to 3.35 mm were used at low speed and under profuse irrigation of saline. The drilled canals were tapped manually.

Implants of the five surface groups were stratified according to implant position. In this way, implants from all five groups were placed within each tibia. The fascia was sutured with Plan Catgut® and the skin with Prolene®. The rabbits were allowed full weight-bearing immediately after surgery. The implant installation procedure was repeated in the right tibia after 2 weeks in group A and after 3 weeks in group B.

Fluorochrome labeling was performed by intravenous injection of calcein green (15 mg/kg; Sigma, USA) after 1 week and alizarin complexone (25 mg/kg; Sigma, USA) after 3 weeks.

Removal torque (RMT) tests of the implants were performed 4 weeks after the second surgery in group A and 9 weeks after the second surgery in group B. Thus, in group A, two healing periods were created, namely 4 and 6 weeks. The corresponding healing periods in the rabbits of group B were 9 and 12 weeks. The RMT procedure included a careful dissection of the soft tissues covering the fixtures. The appendage on the top of the fixture was connected to a digital torque gauge (DTS-485-0-0; Crane Electronics, Hinckley, Leicestershire, England), which allowed the torque to be controlled with an electrical manometer. The torque gauge was oriented parallel to the implant axis and perpendicular to the bone surface. The torque was gradually increased, using a low displacement speed, and the maximum torque shear stress for loosening the implant was recorded.

Immediately after the biomechanical test and the removal of the implants, the animals were sacrificed with an overdose of Hypnorm®. The tibiae were removed, and each implant region (bone bed) was dissected and placed in 10% neutral buffered formalin. Following fixation, each bone bed was embedded in light-curing resin (Technovit 7200 VLC, Kultzer & Co., Germany). The specimens were sectioned with an Exakt sawing machine (Exakt Apparatebau, Norderstedt, Germany) and ground to 10 to 20 μm. The sections were analyzed in a Leica DM-RHC® microscope set (Leica Microsystems, Heidelberg, Germany) for fluorescence microscopy. A light microscopic examination was performed following staining with alizarin red and Stevenels blue.

Differences in torque shear stress between the five surface categories and the healing intervals of each surface category were analyzed using the Student's t-test for paired (within each of the two animal groups: A, B), and unpaired (between group A and group B) observations as well as Pearson's correlation test. The null hypothesis was rejected at $p < .05$.

RESULTS

The results from the surface analysis of the implants are presented in Table 1. The turned implants had the lowest S_a and S_y values, whereas the highest scores were recorded for the TPS implants. The corresponding values for the TiO_2-blasted implants were higher when a larger size of grain particles had been used during surface treatment. When analyzing the six different areas within each implant, it was evident that the TiO_2-blasted implants compared with the TPS implants demonstrated a more homogeneous surface structure.

There was a significant increase of RMT force for all five surface categories between weeks 4 and 6; however, the changes in RMT force between 9 and 12 weeks were not significant (Table 2; Figure 3).

At all four observation intervals (4, 6, 9, and 12 wk), the TPS implants had the highest and the turned implants the lowest RMT values. In general, the differences between the various TiO_2-blasted implants were small, but the group with the largest S_a value had higher RMT values at 6, 9, and 12 weeks than implants with smaller S_a values.

It also was observed that at all examination intervals there was a significant correlation between the S_a and the RMT values ($r = .83$).

Histologic Observations

In all sections representing 4 weeks of healing, the first fluorochrome was identified at the subperiostal and endosteal surfaces of the cortical bone (Figures 4

TABLE 2. Removal Torque Value (Ncm) of Titanium Implants with Different Surface Treatments Inserted in Rabbit Bone for 4, 6, 9, and 12 Weeks

	Group A			Group B		
Surface Treatment/time	n	4 weeks	6 weeks	n	9 weeks	12 weeks
Turned (machined) control	6	8 ± 2	13 ± 3	6	17 ± 4	20 ± 5
TiO_2-blasted						
10–53 μm*	6	13 ± 4	21 ± 6	6	25 ± 4	24 ± 5
63–90 μm*	6	13 ± 3	25 ± 4	6	25 ± 4	27 ± 6
90–125 μm*	6	16 ± 3	29 ± 7	6	36 ± 8	35 ± 4
Titanium plasma-sprayed	6	29* ± 5	46* ± 8	6	54* ± 4	53* ± 6

Connected bar: significant differences between surface groups. *Significant difference compared to all other groups. †Grain size.
n = number of rabbits.
*Grain size.

and 5), whereas the second fluorochrome was observed in the bone tissue between the implant threads (see Figure 5). For biopsies representing 6, 9, and 12 weeks, the appearance of the fluorochromes was less conspicuous (Figure 6).

The light microscopic examination of the 4-week sections revealed that the peri-implant tissue in all surface groups was dominated by woven type bone (Figure 7). However, in the 6-, 9-, and 12-week sections, the content of lamellar bone had increased.

In the sections representing 6, 9, and 12 weeks of healing in TPS and TiO_2-blasted implant categories, ruptures in the zone between old bone and newly formed bone, as well as within the newly formed bone, were frequently observed (see Figure 6).

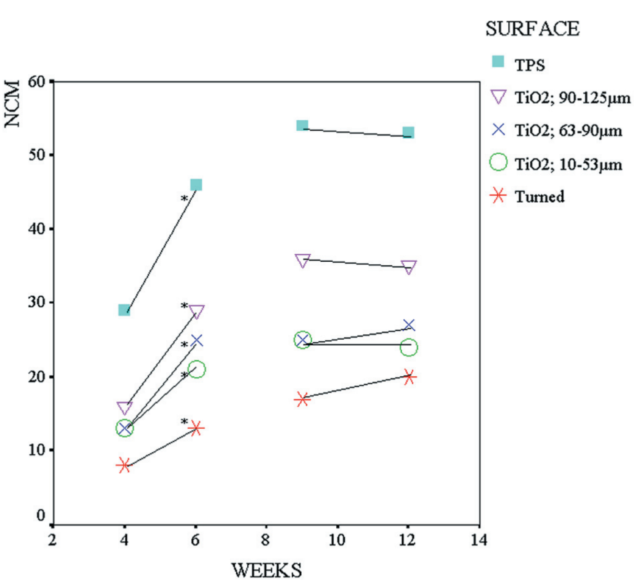

Figure 3. Removal torque value (Ncm) at different healing times (weeks) for the five different implant surface configurations.

DISCUSSION

The finding in the present study, which demonstrates that a relation exists between the surface roughness and the anchorage (RMT) of the implant, is in accordance with observations made in previous animal experiments.[6–10,12–14] It was also observed that at implants with a low S_a value, the removal of the implant caused a

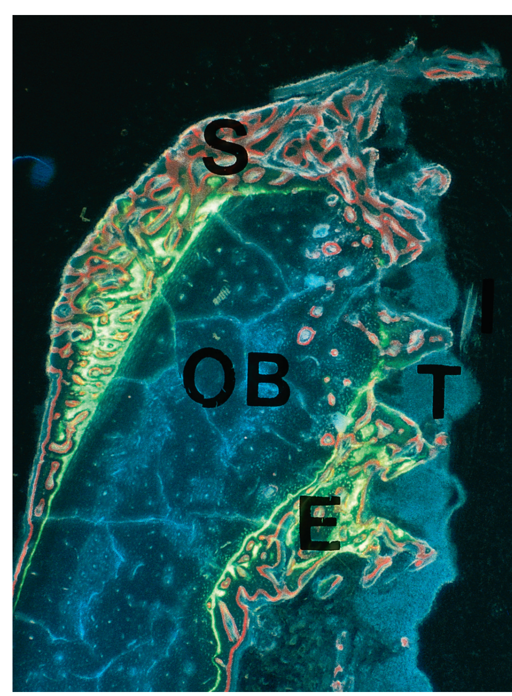

Figure 4. A fluorescence light micrograph of the peri-implant bone adjacent to a TiO_2-blasted (10–53 μm grains) surface 4 weeks after removal of the implant (I). In the “old bone” (OB), only a few fluorochrome lines are observed, whereas a large amount of fluorochrome labeled bone is observed at the subperiostal (S) and endosteal (E) surface of the bone and in the newly formed bone in between the implant threads (T). Original magnification × 10, calcein green (green) and alizarin complexone (red).

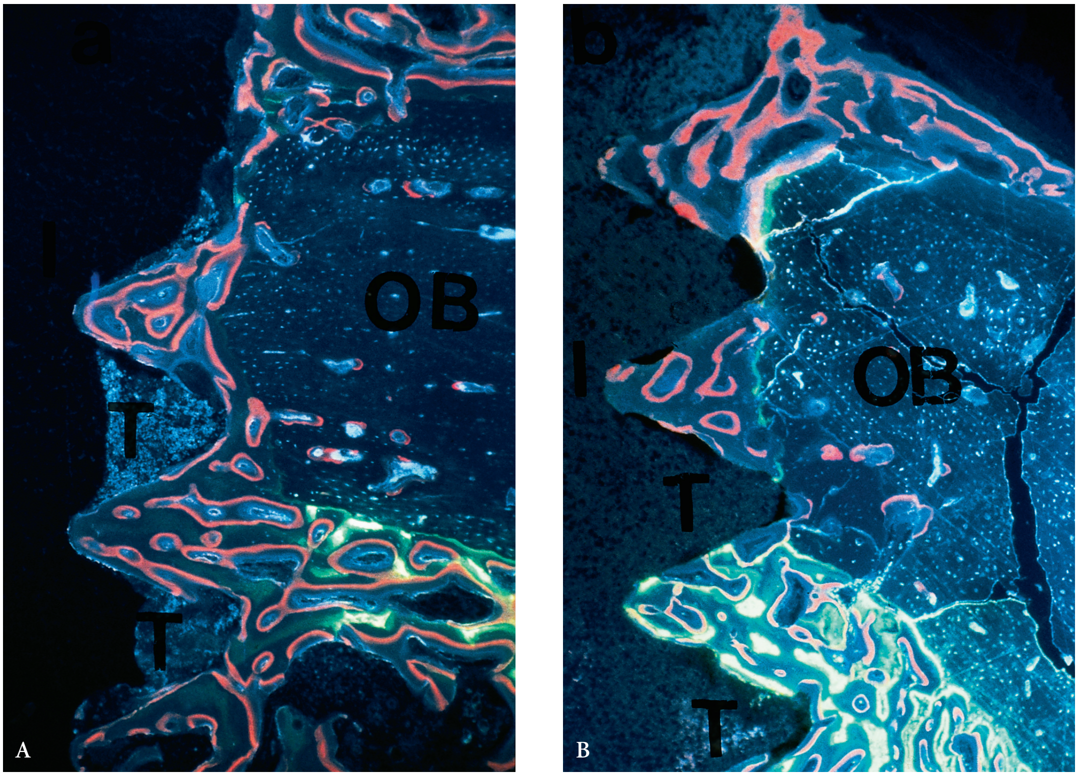

Figure 5. Fluorescence light micrographs of the peri-implant bone 4 weeks after removal of *A,* an implant (I) with TiO_2-blasted (63–90 µm grains) surface; *B,* an implant (I) with turned surface. In both implant sites, alizarin complexone (red) labels are observed in relatively large amounts in the bone between the implant threads (T). Only a few labels are observed in the "old bone" (OB). Original magnification × 25.

rupture in the interface zone between the metal body and the bone, whereas at implants with a higher S_a value, the ruptures also included areas of the peri-implant bone distant from the implant surface.

It has been suggested that there is an upper limit above which an enhanced surface roughness may not further improve bone integration for titanium implants.[11] In two studies,[15,16] screw-shaped titanium implants, blasted with 25-µm and 250-µm particles of Al_2O_3, respectively, were inserted in the tibia and femur of rabbits. No significant differences in terms of RMT values and bone surface areas between the two groups were found after 4 and 12 weeks of healing. This observation is not in agreement with findings made in the present study. Thus, the implants subjected to blasting with grain sizes up to 125 µm demonstrated higher RMT values than the implants blasted with 10- to 53-µm particles. The reason for the difference between the studies by Wennerberg et al and the present experiment,[15,16] as well as previous reports,[9,12] is not yet understood, but may be related to differences in experimental models and examination techniques.

Although RMT analysis has been used in several experiments, the results obtained from different studies may not be directly comparable. This may be attributable to differences in bone anatomy in the implant site, surgical technique used, implant geometry, and specific surface roughness.

In the present study, fluorochrome bone labels were injected after 1 and 3 weeks of healing. From the histologic analyses of the fluorochrome labels it was observed that 1 week after implant placement, mineralized bone had formed at the endosteal and subperiosteal surfaces of the old bone. The location of the second fluorochrome indicated that after 3 weeks new bone had formed between the threads of all implants. There were no obvious differences in the pattern of early bone formation between the five different implant groups.

This observation is consistent with findings reported in a study in rats by Brånemark and colleagues.[17] They found that new bone formation was evident on endosteal, periosteal, and injured surfaces after 4 weeks of healing following implant installation.

In the implant sites representing 6, 9, and 12 weeks in the current study, the peri-implant bone had undergone remodeling and consisted of lamellar bone and bone marrow. This finding is in agreement with previous reports on bone healing around implants placed in the tibia of rabbits.[18] Sennerby and co-workers analyzed bone tissues around implants placed in the tibia and the knee-joints of rabbits after 6, 12, and 21 weeks

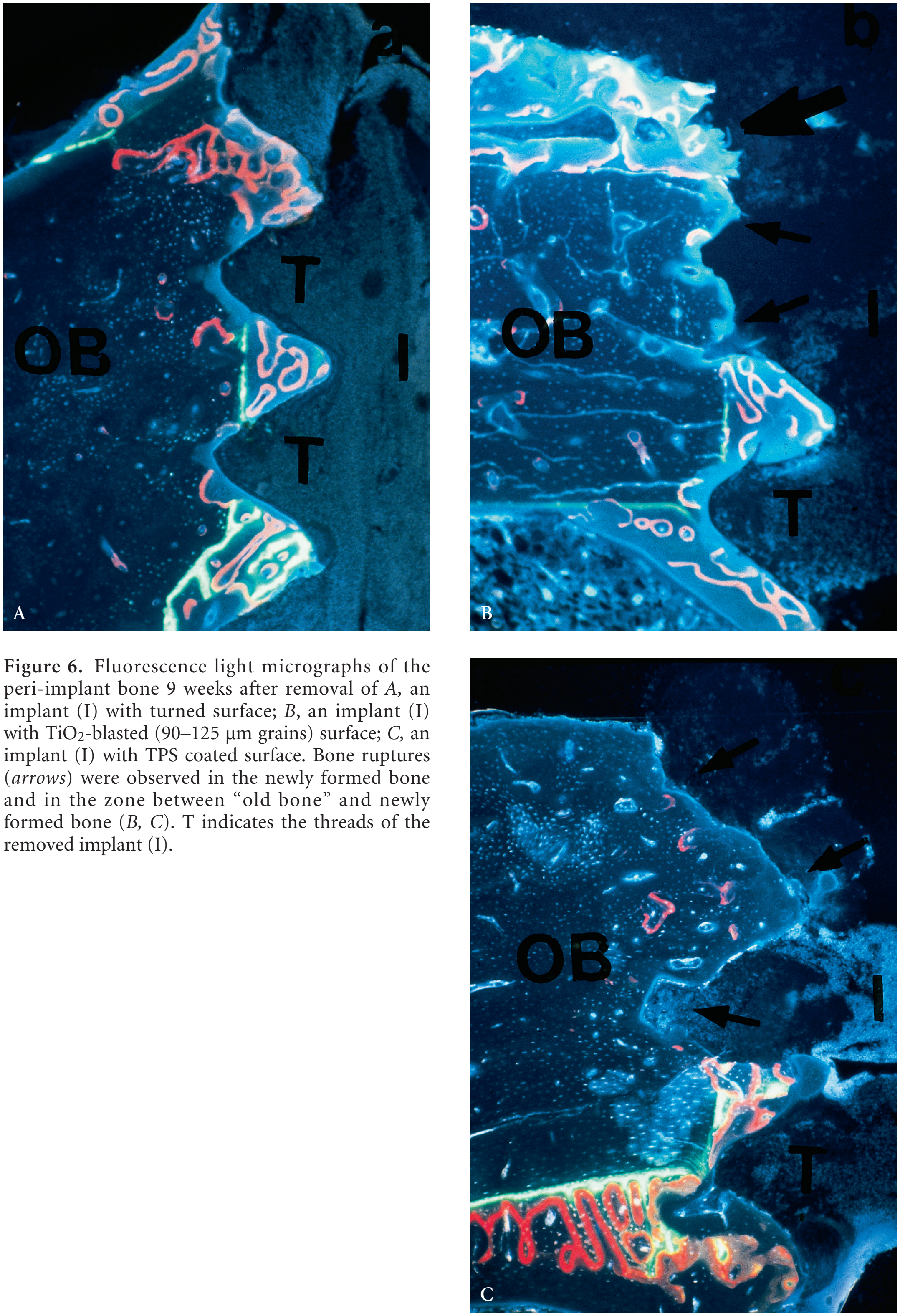

Figure 6. Fluorescence light micrographs of the peri-implant bone 9 weeks after removal of *A*, an implant (I) with turned surface; *B*, an implant (I) with TiO_2-blasted (90–125 μm grains) surface; *C*, an implant (I) with TPS coated surface. Bone ruptures (*arrows*) were observed in the newly formed bone and in the zone between "old bone" and newly formed bone (*B, C*). T indicates the threads of the removed implant (I).

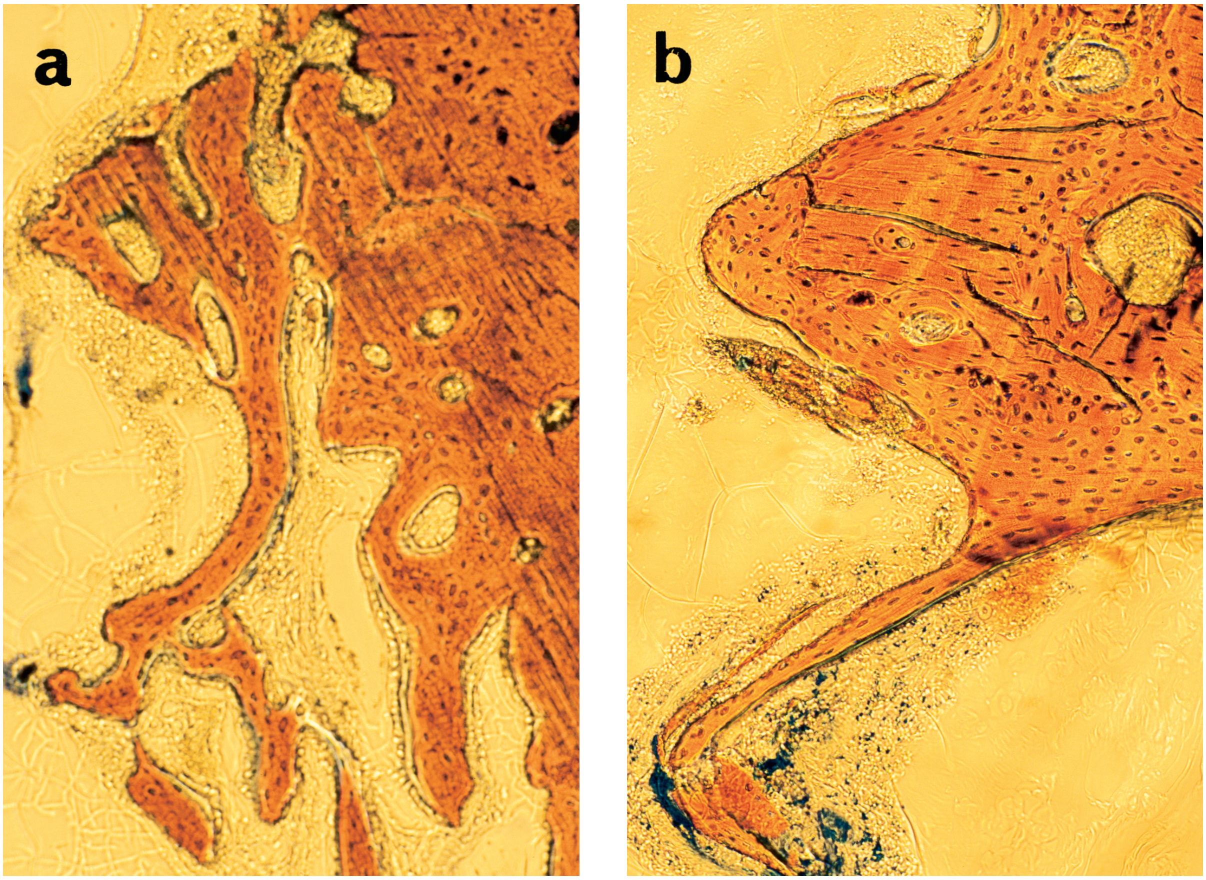

Figure 7. Ground sections of the peri-implant bone at 4 weeks (*A*) and at 12 weeks (*B*) after the removal of an implant (I) with a turned surface.

of healing.[18] They reported that after 6 weeks the hard tissue between the upper threads of the implants contained well-organized cortical bone.

In the present study, it was observed that the resistance to torque for all five implant groups increased over time. This finding is in agreement with previous animal experiments.[17,19] Johansson and Albrektsson installed implants in loop-eared rabbits and reported that RMT values assessed at 3, 4, and 12 weeks increased from 10.8 Ncm to 68 Ncm.[19] Brånemark and colleagues,[17] in a rat experiment, found a significantly increased torsional capacity from 4 to 16 weeks of healing. On the other hand, Sennerby and colleagues,[18] in their rabbit study, failed to detect any difference in RMT values for tibial implants removed after 6, 12, and 21 weeks of healing. It may be argued that the observation intervals in the study by Sennerby et al reflect later healing sequences than the study by Brånemark et al and the present experiment.[17,18]

In the current study, the rupture in the peri-implant bone that occurred as a result of the removal of the implant was located in different areas in the various implant surface groups. In sites representing the turned implants, the ruptures were identified in the interface between the implant and the bone, whereas in the sites representing rough implant surfaces, the ruptures also occurred in the peri-implant bone distant to the implant–bone interface. Sennerby et al,[18] in the rabbit study, analyzed implant sites in which implants were unscrewed after 12 months of healing. They reported that the rupture occurred close to the implant surface and not within the bone, and that no tissue remained on the implant surface. This finding is consistent with observations made in the present study with regard to the turned implant group.

CONCLUSION

The present study demonstrated that a clear relation exists between surface roughness, described in S_a values, and implant anchorage assessed by RMT measurements. Anchorage appeared to increase with the maturation of bone tissue during healing.

ACKNOWLEDGMENTS

Nils Kofod from the Technical University of Denmark is gratefully acknowledged for the surface roughness tests, and Astra Tech AB, Sweden, and Institute Straumann AG, Switzerland, are thanked for the surface treatments.

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