# Removal Torque and Histomorphometric Investigation of 4 Different Titanium Surfaces: An Experimental Study in the Rabbit Tibia

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*This study presents a histomorphometric and biomechanical comparison of bone response to commercially pure titanium screws with 4 different types of surface topographies placed in the tibial metaphysis of 12 rabbits. Each rabbit had 4 implants placed, 2 in each tibia. The 4 surface topographies were a machined surface, a grit-blasted surface, a plasma-sprayed surface, and an acid-etched (Osseotite) surface. After a healing period of 5 weeks, histomorphometric and removal torque data revealed a significantly higher percentage of bone-to-implant contact and removal torque for acid-etched implants compared to machined, blasted, and plasma-sprayed implants. Within the limits of this short-term experimental study, the results indicated that micro-rough titanium surfaces obtained with acid-etching procedures achieved a 33% greater bone-to-implant contact over machined titanium surfaces with an abutment-type roughness and provided enhanced mechanical interlocking.* (INT J ORAL MAXILLOFAC IMPLANTS 2000; 15:668–674)

**Key words:** *dental acid etching, endosseous dental implants, histomorphometry, surface properties, titanium*

A crucial factor in the successful osseointegration of endosseous implants is a favorable interaction between the implant geometry and surface texture and the tissues at the bone site.[1] Various procedures have been tested to improve the anchorage strength and mechanical interlocking of root-form dental implants by modifying implant characteristics, especially the implant surface texture, by roughening,[2–7] coating,[8,9] or chemical treatment.[10–15] Recent studies have reported increased mechanical interlocking between bone and the micro-rough surface of sandblasted and acid-etched implants.[12,16] Acid-etching alone has been evaluated by Klokkevold and coworkers,[17] who demonstrated superior resistance to reverse torque removal of acid-etched surfaces as compared to machined surfaces. The purpose of the present study was to further evaluate bone tissue reactions around acid-etched (Osseotite, Implant Innovations Inc, Palm Beach Gardens, FL) threaded implants placed in the rabbit tibia using 2 types of quantitative tests. Bone response was quantified by light microscopic morphometry and anchorage was analyzed by measuring the removal torque. The anchorage of acid-etched implants was compared with conventional machine-produced, grit-blasted, and plasma-sprayed titanium screws.

## MATERIALS AND METHODS

### Animals and Anesthesia

Twelve adult New Zealand white rabbits weighing 3.5 to 4 kg were used in this study. The animals were anesthetized using intramuscular injections of

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ketamine (Ketalar, Parke-Davis S.p.A., Milano, Italy, 44 mg/kg of body weight) and xylazine (Rompun, Bayer AG, Leverkusen, Germany, 6 to 8 mg/kg of body weight). Prior to surgery, 1.8 mL of lidocaine 2% (Xylocaine, Astra, Södertälje, Sweden) were injected locally into the surgical sites. Postoperatively, the animals received antibiotics (Penovet, Boehringer Ingelheim Danmark A/S, Copenhagen, Denmark) at a dose of 0.3 mL per animal and analgesics (Temgesic, Reckitt and Coleman, Hull, England) at 0.05 mg/kg of body weight for 3 days and were allowed full weight-bearing and movement postsurgically. Five weeks after surgery, the animals were sacrificed using an overdose of carbon dioxide.

### Surface Treatment and Characterization of Implants

A total of 48 custom-made, screw-shaped, commercially pure titanium implants (Implant Innovations Inc) with a length of 4 mm, an outer diameter of 3.75 mm, and a pitch height of 0.5 mm was used in this study. The implants were divided into 4 groups:

- Group A: 12 implants left as-machined
- Group B: 12 implants blasted with commercially pure titanium dioxide particles (10 to 60 µm in size) at a pressure of 70 psi
- Group C: 12 implants plasma spray-coated using commercially pure titanium with a coating thickness of 50 to 70 µm
- Group D: 12 implants acid-etched using 2 acid exposures at high temperature, the first exposure with hydrofluoric acid and the second with a combination of hydrochloric and sulfuric acid (Osseotite)

Qualitative and quantitative characterization of the 4 different surface topographies was carried out using a vertically scanning interference microscope (WYKO NT-2000, WYKO Corporation, Tucson, AZ). In 3 samples from each of the 4 types of surface topographies, 3 threads were selected at random and scanned along their circumference in 4 different areas, yielding 12 measurements for each surface topography. The measuring area was 200 × 250 µm for all measurements. Three height parameters, $R_a$, $R_q$, and $R_z$, were used for quantitative characterization of the surface roughness. $R_a$ describes the arithmetic mean of the departures of the roughness profile from the midline. $R_q$ is the root mean square parameter corresponding to $R_a$, and $R_z$ measures the average height difference between the 5 highest peaks and the 5 lowest valleys.

The implants were cleaned, packaged, and further sterilized by gamma radiation.

### Surgical Technique and Implant Placement

Prior to surgery, the skin was cleaned with a mixture of iodine and 70% ethanol. The tibial metaphysis was exposed by incisions through the skin, fascia, and periosteum. By intermittent drilling using low rotary speed and copious saline irrigation, 2 holes were drilled 7 mm apart in the central portion of each tibia and sequentially enlarged to 3.2 mm. The implants were gently screwed into place, without tapping the sites, until the implant shoulder was level with the bone surface. All implants penetrated the first cortical layer only, never engaging the opposite cortical side. Each rabbit received 4 implants, 1 each of the different surface topographies (groups A, B, C, and D), which were randomly assigned to their implantation sites. Titanium cover screws were placed on the implants, and the fascia and skin were closed in separate layers using resorbable sutures.

### Torque Measurements and Histologic Preparation of Specimens

Five weeks postsurgically, 7 randomly chosen rabbits were anesthetized. The implant sites were exposed, and the cover screws and the bone and soft tissues that had formed on top of the implants were carefully removed. Subsequently, the force needed to unscrew the implants (n = 28) was measured using a torque gauge manometer (Tohnichi, Model 6 BTG-N, Tokyo, Japan). The implants were subjected to slowly increasing torque until loosening was detected, and the peak torque value was measured when rupture occurred between implant and bone.

The remaining 5 animals were sacrificed without subjecting the implants to removal torque, and the specimens (including implants; n = 20) and surrounding tissues were washed in saline solution and fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.15 mol/L cacodylate buffer at 4°C and pH 7.4. The specimens were further dehydrated in ascending concentrations of alcohol rinses and infiltrated with glycolmethacrylate resin (Technovit 7200 VLC, Kulzer & Co, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally at about 100 µm and ground to a final thickness of about 30 µm (Precise 1 Automated System, Precise, Pescara, Italy) as described by Donath.[18] Two sections were obtained for each implant and stained with acid fuchsin and toluidine blue. The histomorphometric analysis was performed using a Leitz Laborlux S microscope and the Leitz Microvid equipment (Leitz, Wetzlar, Germany) connected to a personal computer. The percentage of bone-to-implant contact around all threads throughout the length of the implant body was calculated using a 10× objective.

**Figs 1a to 1d** Photomicrographs of scanning electron microscopy of the 4 different surface topographies.

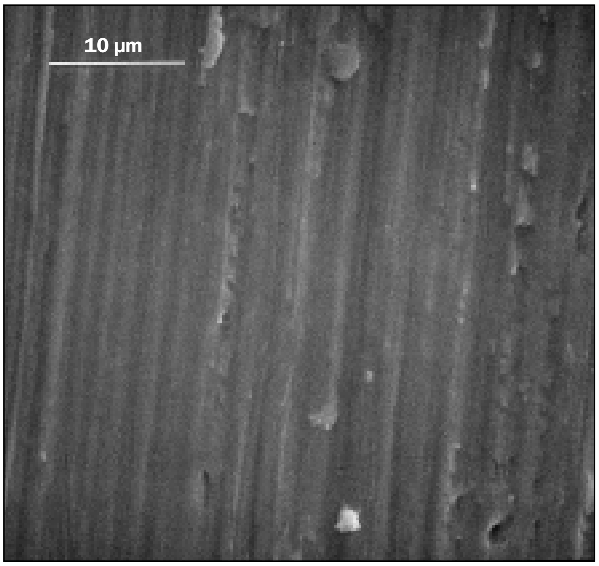



**Fig 1a** The machined surface reveals the standard machining lines and relatively smooth surface.

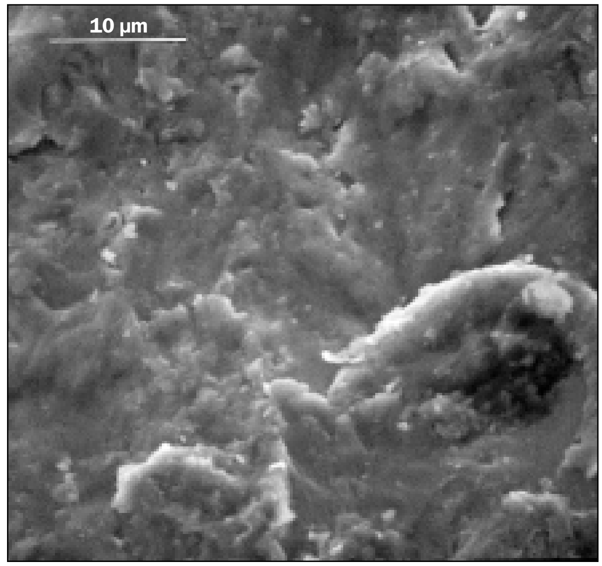



**Fig 1b** The grit-blasted surface is rougher than the machined surface but has an irregular pattern.

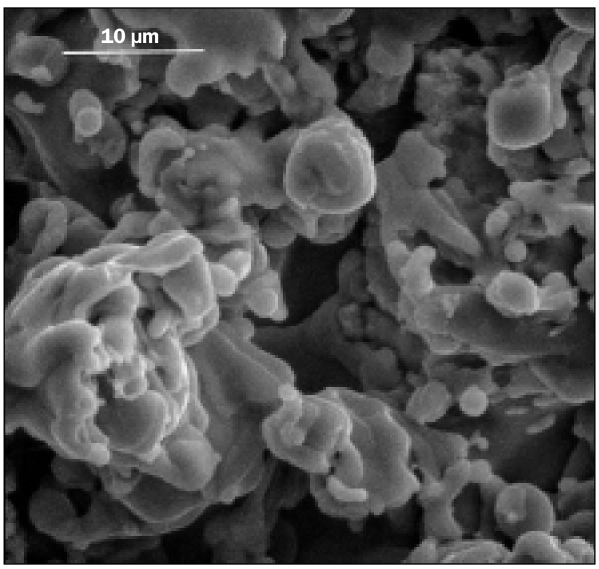



**Fig 1c** The titanium plasma-sprayed surface has a globular appearance with relatively flat surfaces mixed with deeper clefts.

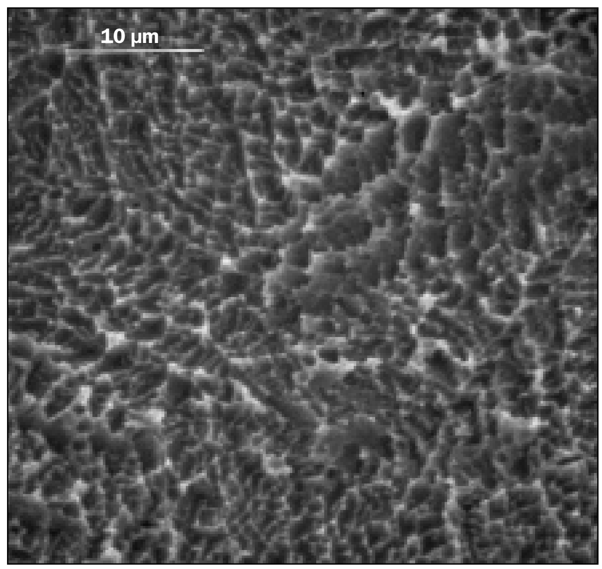



**Fig 1d** The Osseotite surface has a uniform micro-roughness with small openings on the surface.

### Statistical Analysis

Mean values of bone-to-implant contact and removal torque were calculated and subjected to a 2-tailed analysis of variance to test for significant differences between the 4 investigated surfaces. The first factor was surface topography and the second the block (ie, subject) factor. Statistical testing was carried out at the 5% significance level.

## RESULTS

### Topographic Evaluation

The qualitative SEM analysis (Figs 1a to 1d) and the surface roughness measurements (Table 1) demonstrated the different surface topographies found in the 4 implant groups. The machined specimens showed the smoothest surfaces, while the titanium plasma-sprayed implants had the roughest surface structure. The machined surface showed an

**Table 1 Mean Surface Roughness (in µm) of the 4 Different Surface Treatments, Characterized by Height and Spatial Parameters**

| Surface treatment | $R_a$ (SD) | $R_q$ (SD) | $R_z$ (SD) |
|---|---|---|---|
| Machined | 0.29 (0.07) | 0.37 (0.07) | 2.90 (0.66) |
| Osseotite (acid-etched) | 0.62 (0.17) | 0.79 (0.23) | 5.45 (1.57) |
| Grit-blasted | 1.26 (0.29) | 1.53 (0.35) | 6.54 (1.16) |
| Plasma-sprayed | 9.10 (4.86) | 12.70 (4.60) | 81.60 (23.30) |

$R_a$ = arithmetic mean of the departures of the roughness profile from the midline; $R_q$ = root mean square parameter corresponding to $R_a$; $R_z$ = average height difference between the 5 highest peaks and the 5 lowest valleys.

**Table 2 Removal Torque (in Ncm) 5 Weeks after Implant Placement**

| Rabbit no. | Machined | Acid-etched | Grit-blasted | Plasma-sprayed |
|---|---|---|---|---|
| 1 | 21 | 35 | 26 | 25 |
| 2 | 30 | 40 | 29 | 30 |
| 3 | 27 | 38 | 25 | 32 |
| 4 | 23 | 45 | 28 | 29 |
| 5 | 22 | 42 | 25 | 30 |
| 6 | 28 | 39 | 27 | 31 |
| 7 | 26 | 47 | 28 | 30 |
| Mean | 25.28 | 40.85 | 26.85 | 29.57 |
| SD | 3.35 | 4.14 | 1.57 | 2.22 |

average surface roughness ($R_a$) of 0.29 µm; corresponding values for the Osseotite, $TiO_2$-blasted, and plasma-sprayed implants were 0.62 µm, 1.26 µm, and 9.10 µm, respectively.

## Removal Torque Measurements

Five weeks after implant placement, the average removal torque was 25.28 ± 3.35 Ncm for the machined implants, 26.85 ± 1.57 Ncm for the grit-blasted implants, 29.57 ± 2.22 Ncm for the plasma-sprayed implants, and 40.85 ± 4.14 Ncm for the acid-etched implants. The removal torque results are summarized in Table 2. The torque measurements yielded statistically significant differences between the acid-etched group and the remaining 3 groups ($P < .05$). The highest removal torque corresponded to the acid-etched implants, while the lowest was demonstrated by the machined implants. No significant differences in removal torque were found between the plasma-sprayed and grit-blasted surfaces or between the grit-blasted and machined surfaces. However, higher torque was needed to unscrew plasma-sprayed implants compared to the torque needed for the machined implants.

## Histomorphometric Evaluation

Microscopically, all 20 implants were well integrated into bone. The implants were in contact with predominantly cortical bone along the upper threads in the cortical region, while the threads in the bone marrow were in contact with either newly formed bone or with normal marrow tissue. A demarcation line was consistently seen between the newly formed bone and the old bone tissue (Fig 2). Qualitative histologic differences among the various surface topographies were not seen.

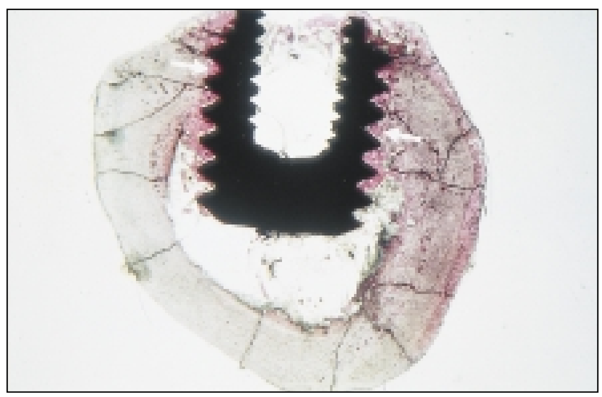
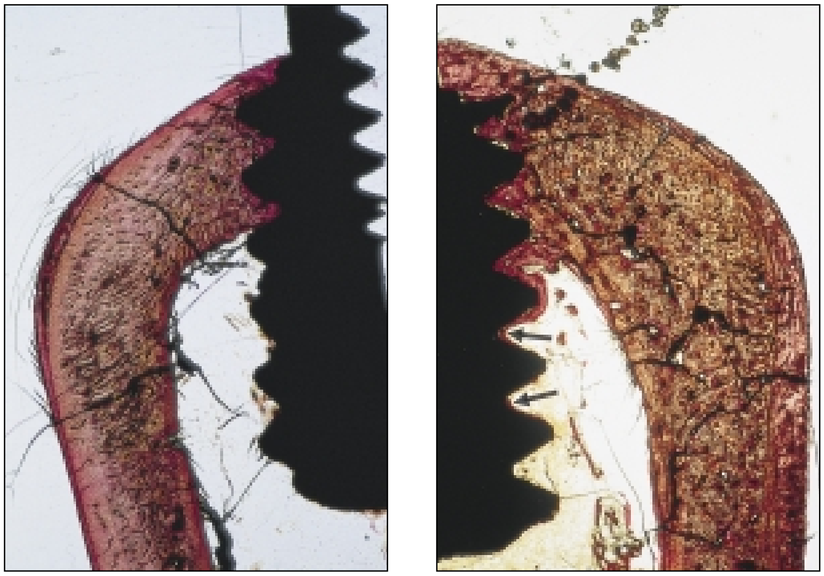


**Fig 2** Thirty-micron ground section of an acid-etched implant 5 weeks after placement in the rabbit tibia. A clear demarcation line (*arrow*) is evident between the old bone (*pale purple*) and newly formed bone (*dark purple*). Bone-to-implant contact can be observed along the middle and lower threads of the implant (acid fuchsin and toluidine blue; original magnification ×2.5).



**Figs 3a and 3b** Thirty-micron ground sections of (*left*) a plasma-sprayed implant and (*right*) an acid-etched implant after 5 weeks of implantation in the rabbit tibia. Note bone on the surface of the middle threads of the acid-etched implant (*arrows*), while only the first 2 threads of the plasma-sprayed implant are occupied by bone (acid fuchsin and toluidine blue; original magnification ×6).

**Table 3 Percentage of Bone-Implant Contact 5 Weeks after Implant Placement**

| Rabbit no. | Machined | Acid-etched | Grit-blasted | Plasma-sprayed |
|---|---|---|---|---|
| 8 | 40 | 66 | 45 | 43 |
| 9 | 45 | 68 | 61 | 60 |
| 10 | 51 | 64 | 50 | 55 |
| 11 | 45 | 76 | 47 | 53 |
| 12 | 62 | 88 | 71 | 73 |
| Mean | 48.60 | 72.40 | 54.80 | 56.80 |
| SD | 8.44 | 9.83 | 10.96 | 10.96 |

The histomorphometric findings paralleled the removal torque data. A mean percentage of bone-to-implant contact of 48.60 ± 8.44% was found for the machined surfaces, 54.80 ± 10.96% for the blasted surfaces, 56.80 ± 10.96% for the plasma-sprayed surfaces, and 72.40 ± 9.83% for the acid-etched surfaces (Figs 3a and 3b). The histomorphometric analyses are summarized in Table 3. The analysis of histomorphometric data revealed statistically significant differences to the advantage of the acid-etched group ($P < .05$). There were no statistically significant differences in the percentage of bone-to-implant contact length between plasma-sprayed and grit-blasted surfaces or between the grit-blasted and machined surfaces. However, the plasma-sprayed surfaces demonstrated statistically significantly higher bone-to-implant contact than the machined specimens.

## DISCUSSION

The present study showed that acid-etched (Osseotite) titanium implants achieved a higher degree of bone-to-implant contact compared to machined, blasted, and plasma-sprayed implants. One interesting finding with the acid-etched implants in this study was the presence of compact bone along the middle and lower threads initially located in cancellous bone. In the other 3 groups of surface topographies, compact bone was in contact mainly with the first threads of the implants and failed in most instances to reach the lower threads. These findings correlate with the results of a human histologic investigation[19] that reported a mean percentage of bone-to-implant contact of 33.98 ± 31.04% for machined surfaces, versus 72.96 ± 25.13% for Osseotite surfaces, 6 months after placement of the test implants in the tuberosity region. In that study,[19] the authors reported evidence of remodeled bone along the Osseotite surface, particularly in areas that were previously located in marrow spaces. Furthermore, in vivo experiments by Davies and Diziedgie (unpublished data) investigating bone growth into hollow test chambers lined with acid-etched (Osseotite) and machined surfaces demonstrated that acid-etched surfaces supported more and closer bone growth. At the cellular level, these differences between Osseotite and machined surfaces may be explained by the micro-rough topography of acid-etched surfaces, which could favorably affect angiogenesis as well as cellular migratory behavior, cell alignment, orientation, and attachment, and finally cell activity and function.[20]

Acid-etched implants yielded higher removal torque values than the 3 remaining surface topographies; the average removal torque of the acid-etched implants was 38%, 34%, and 28% higher than the machined, blasted, and plasma-sprayed implants, respectively. These results are in agreement with those of Klokkevold and colleagues,[17] who reported that the chemically etched implant surfaces (Osseotite) conferred a resistance to reverse torque removal in the rabbit femur that was 4 times greater than that of machined surfaces 2 months post-placement. The mean torque values obtained in the present study are comparable with mean torque values observed for similar healing periods in other studies using threaded implants of similar dimensions in the rabbit tibia[21]; however, they are higher than the torque measurements reported by Klokkevold and colleagues.[17] The higher torque resistance demonstrated in this investigation can be attributed to differences in implant size and structural differences at the bone implantation sites. The greater removal torque values achieved with the acid-etched group may be related primarily to the higher bone-to-implant contact. A positive correlation between the degree of bone in contact with the implant and removal torque was reported by Johansson and Albrektsson.[22] When it is considered that all 4 surface topographies studied in this investigation had a similar degree of bone-to-implant contact at the first threads (unreported data), it may be suggested that the higher removal torque measurements seen with the acid-etched implants could be related to the increased degree of bone contact with the implant surface in the area of cancellous bone.

Several studies have reported that rough implant surfaces of varying topography generally demonstrate increased bone apposition and better bone anchorage when compared to machined surfaces.[3,5,23,24] In this study, no significant differences were evidenced between grit-blasted and machined surfaces with respect to bone-to-implant contact and removal torque values. There are inherent difficulties in comparing biologic and biomechanical responses to biomaterials with specific surface roughness characterization. The differences between the present observations and those previously reported may be attributed to differences in length of the follow-up periods, site variations, and implant surface roughness parameters. Other factors such as initial implant stability,[24] implant cleaning and sterilization procedures,[25] and orientation of the surface structure[23] may be crucial factors in bone tissue response. The less favorable results obtained in the present study with grit-blasted and plasma-sprayed implants may be accounted for by the fact that very small variations of the surface pattern in the micrometer range can elicit different cellular responses.[26–28]

## CONCLUSIONS

Within the limits of this 5-week experimental study, it may be stated that the overall pattern of implants treated with acid-etching procedures and characterized by an average roughness of 0.62 µm resulted in a significantly higher percentage of bone-to-implant contact and removal torque when compared to machined, grit-blasted, and plasma-sprayed implants. The present investigation did not demonstrate any statistically significant differences in the percentage of bone-to-implant contact between plasma-sprayed and grit-blasted surfaces or between grit-blasted and machined surfaces.

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