Histologic and Histomorphometric Analysis of the Bone Response to Machined and Sandblasted Titanium Implants: An Experimental Study in Rabbits

Adriano Piattelli, MD, DDS*/Licia Manzon, MD, DDS**/Antonio Scarano, DDS***/
Michele Paolantonio, MD, DDS****/Maurizio Piattelli, MD, DDS****

The aim of this study was to make a comparative analysis between the bone response to machined and sandblasted implants. The sandblasting was done with 150-µm aluminum oxide particles. Under scanning electron microscopic examination, the machined implants presented typical machining grooves, while a very rough, highly irregular surface with depressions and indentations was present on the sandblasted implants. Light microscopy showed a different bone growth pattern on machined (implantopetal growth) and sandblasted (implantofugal growth) implants. No negative effects on the rate of bone growth were observed in spite of the presence of aluminum ions. The histomorphometric analysis showed that sandblasted implants presented, from the third week onwards, a significantly higher contact percentage ($P < .0001$). These values could point to higher osteoconductivity as a result of the higher surface roughness of sandblasted surfaces.
(INT J ORAL MAXILLOFAC IMPLANTS 1998;13:805–810)

Key words: bone growth, sandblasting, surface roughness, titanium implants

The surface morphology of dental implants has received increasing attention in recent years.[1–2] Macroscopic characteristics of the implant surface influence cellular events present at the bone-biomaterial interface and are critical for the long-term survival of the implant.[2–5] The cells have been demonstrated to be sensitive to microtopography.[4] Osteoblasts showed an initial attachment to rough titanium (Ti) surfaces,[2,4] and further Ti surface roughness has been shown to affect osteoblast proliferation and differentiation.[4] Macrophages showed rugophilia, ie, affinity for rough surfaces, while, on the contrary, fibroblasts failed to adhere to rough surfaces.[2] Moreover, matrix vesicle alkaline phosphatase-specific activity was enhanced by surface roughness.[4,6]

Surface blasting is a process by which metal surfaces are treated with different types of materials (aluminum oxide, titanium oxide, etc) to provide an irregular surface.[7,8] Blasted surfaces show a rough irregular topography with numerous randomly oriented rough features.[3] Surface blasting, in addition to increasing the surface roughness, removes surface contaminants and increases surface reactivity of the metal.[9] Also, a significantly higher torque was required for the removal of blasted implants.[9] Blasted implants present a significantly longer bone-implant interface than smooth ones.[8] It is also possible that cells located on rougher surfaces may stay longer in a proliferative state before further differentiation.[4] Some blasted implants have shown the presence of areas of direct bone apposition on the metal surface with no space present between the new bone and implant surface.[8] Surface blasting can increase the rate and amount of bone formation on the implant surface.[8] No negative effects from aluminum (Al) ions were seen on the peri-implant bone tissues,

*Professor of Oral Pathology and Medicine, Dental School University of Chieti, Chieti, Italy; Honorary Senior Lecturer, Eastman Dental Institute for Oral Health Care Sciences, London, United Kingdom.
**Professor of Dental Materials, Dental School, University of Rome, Rome, Italy.
***Research Fellow, Dental School, University of Chieti, Chieti, Italy.
****Researcher, Dental School, University of Chieti, Chieti, Italy.

Reprint requests: *Dr Adriano Piattelli, Via F. Sciucchi 63, 66100 Chieti, Italy. Fax: 011-39-871-3554076. E-mail: apiattelli@unich.it*

COPYRIGHT © 2000 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

even if blasted surfaces contained a significant amount of aluminum, most probably because with blasted implants only a limited and transient release of Al ions occurs.[3] Moreover, 25-µm titanium oxide (TiO_2) and aluminum oxide (Al_2O_3) showed very similar surface structures quantitatively as well as qualitatively.[9] On the other hand, surface roughness modifications can have a negative influence on the microcomposition, crystallographic structure, and surface energy and, subsequently, on the biologic response of the implant.[7]

The aim of the present study was to compare the responses in implants with a machined surface and implants with a surface sandblasted with 150-µm Al_2O_3 particles.

Materials and Methods

Threaded, machined, and sandblasted (with 150-µm Al_2O_3 particles) grade 3 commercially pure titanium screw-shaped implants (Restore, Lifecore, Chaska, MN) were used in this study. The blast pressure was 20 psi and the blast time was 15 seconds. The particle size of the Al_2O_3 was 100 mesh (approximately 149 µm). Particle distribution was accomplished as follows: a 100-mesh (or 149-µm) material was used initially, and 100% of the particles had to pass through a 70-mesh (210-µm) sieve. The particles had then to pass through a 100-mesh sieve, where the maximum number of particles that was larger than 100 mesh was 20%. Then the particles were passed through a 120-mesh (125-µm) sieve, and a minimum of 40% needed to go through the sieve. Subsequently, using a 120- to 140-mesh sieve, a minimum of 65% of the particles had to go through, and then using a 200-mesh sieve a maximum of 3% of particles had to pass through.

Forty-five New Zealand white mature male rabbits were used for this study. The implants were placed into the articular femoral knee joint according to a previously described technique.[10] Each rabbit received 2 implants—1 test (sandblasted) and 1 control (machined).

A total of 90 implants (45 control and 45 test) were placed. The rabbits were anesthetized with intramuscular injections of fluanizone (.7 mg/kg body weight) and diazepam (1.5 mg/kg body weight), and anesthesia was administered locally using 1 ml of 2% lidocaine/adrenalin solution. A skin incision with a periosteal flap was used to expose the articular surface. Preparation of the bone site was done with burs under generous irrigation with saline. The implant placement was performed by hand. The periosteum and fascia were sutured with catgut and the skin was sutured with silk.

Three rabbits presented postoperative complications, and death occurred in the first postoperative week. Three test implants and 3 control implants were therefore lost; these implants were discarded. Postoperatively, the animals received intramuscular injections of penicillin (2 million IU/5 ml; .1 ml/kg b.wt.). Eight animals were sacrificed with an overdose of intravenous pentobarbital after 1, 2, 3, and 4 weeks; 10 rabbits were sacrificed after 8 weeks.

A total of 84 implants were retrieved. The implants and surrounding tissues were washed in saline solution and immediately fixed in 4% paraformaldehyde and .1% glutaraldehyde in .15 M cacodylate buffer at 4°C at a pH of 7.4 to be processed for histology. The specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).[11] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycol-methacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disc at about 150 µm and ground down to about 30 µm with a specially designed grinding machine.

A total of 3 slides were created for each implant. The slides were stained with acid and basic fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Leitz Laborlux microscope (Leitz, Wetzlar, Germany). The histochemical analysis was done according to a previously published protocol.[12] The histomorphometry was done with a Microvid system (Leitz, Wetzlar, Germany) connected to an IBM personal computer. Three test and 3 control implants were analyzed under a Cambridge 360 scanning electron microscope (Cambridge Instruments, Cambridge, United Kingdom). Roughness measurements were made using a Mitotoyo Surftest 211 Profilometer (Mitotoyo, Tokyo, Japan); 3 readings were obtained for each surface and averaged. A total of 10 implants (5 machined and 5 sandblasted) were analyzed.

Data Analysis. The implant represented the unit of analysis. Modifications of the percentages of bone contact throughout the study period in each experimental group were treated by analysis of variance (ANOVA). The statistical significance of the differences in the percentage of bone contact between test and control implants was assessed by Student's t-test for unpaired samples. Values of $P < .05$ were considered to be statistically significant. Actual P values were not given because an "exact test" was not used.

Copyright © 2000 by Quintessence Publishing Co, Inc. Printing of this document is restricted to personal use only. No part of this article may be reproduced or transmitted in any form without written permission from the publisher.

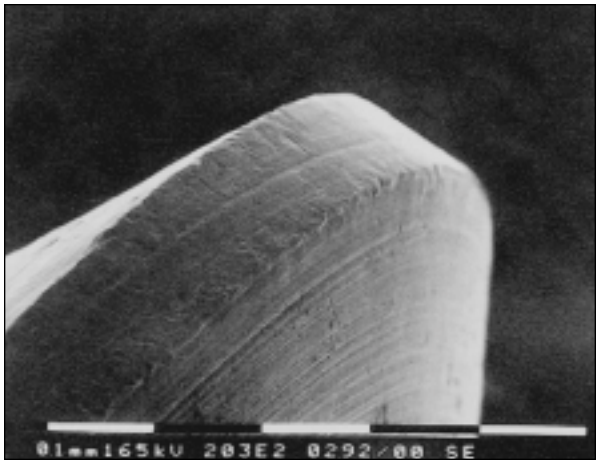

Fig 1 SEM of a machined implant. Typical machining grooves are present on the implant surface.

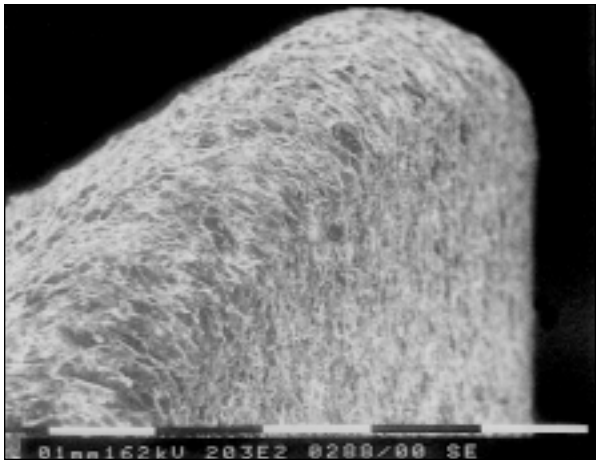

Fig 2 SEM of a sandblasted implant. Depressions and indentations are present on the implant surface.

Results

The microprobe analysis showed the presence of aluminum particles on the surface of the sandblasted implants.

Scanning Electron Microscopy of Machined Implants. Typical machining grooves produced by the manufacturing instruments were observed on the surface of the implants (Fig 1).

Scanning Electron Microscopy of Sandblasted Implants. The implant surface appeared glazed, and a very rough surface produced by the blasting procedure was observed. The surface was highly irregular, with many depressions and small indentations (Fig 2).

Light Microscopy. All implants were evaluated under light microscopy 1 week, 2 weeks, 3 weeks, 4 weeks, and 8 weeks after placement.

Machined Implants at 1 Week. At low magnification, the presence of bone trabeculae near the implant surface was observed. At higher magnification, many actively secreting alkaline phosphate (ALP+) osteoblasts were observed. In many areas not yet mineralized, matrix was present.

Sandblasted Implants at 1 Week. Many ALP+ osteoblasts were present and in direct contact with the implant surface. In other areas of the implant perimeter, the formation of osteoid matrix directly on the implant surface was observed.

Machined Implants at 2 Weeks. An increased number of bone trabeculae were observed. Bone trabeculae were present near the implant surface (Fig 3). Many ALP+ osteoblasts were present, and they were secreting osteoid matrix toward the implant surface, ie, in an implantopetal direction.

Sandblasted Implants at 2 Weeks. The bone was in close contact with the titanium surface of the implant (Fig 4). In some areas the osteoid matrix was undergoing mineralization. Near to the implant there was, in some instances, a double layer of osteoblasts; one layer was depositing osteoid matrix directly on the implant surface, while the other was forming the matrix near the implant surface.

Machined Implants at 3 Weeks. A higher quantity of bone and ALP+ osteoblasts around the implants were observed. A 2- to 5-µm gap between newly-formed bone and the implant surface was observed (Fig 5).

Sandblasted Implants at 3 Weeks. An increased number of ALP+ osteoblasts were observed. Many osteoblasts were located directly on the implant surface, while in other regions, osteoid matrix and bone were present. No gap was present between the bone and the implant.

Machined Implants at 4 Weeks. Mature bone with few marrow spaces was present. A sharp decrease in the number of ALP+ osteoblasts was observed. In only a few areas was bone in direct contact with the implant.

Sandblasted Implants at 4 Weeks. A sharp decrease in the number of ALP+ osteoblasts was observed; these cells were present only in a few areas of the interface. Mature bone and marrow spaces were present in other areas of the interface (Fig 6).

Machined Implants at 8 Weeks. The quantity of bone was similar to that observed at 4 weeks. In only a few areas were ALP+ osteoblasts observed. Mature bone appeared in direct contact with the implant surface (Fig 7), but in many areas nonmineralized osteoid matrix was interposed between mineralized bone and the implant surface.

COPYRIGHT © 2000 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

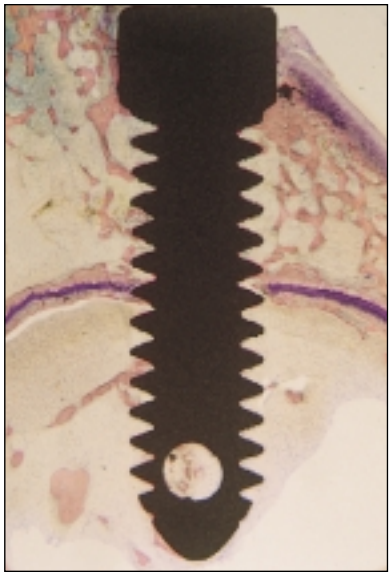

Fig 3 Machined implant at 2 weeks. Bone trabeculae near the implant surface are present (acid fuchsin-toluidine blue; magnification ×20).

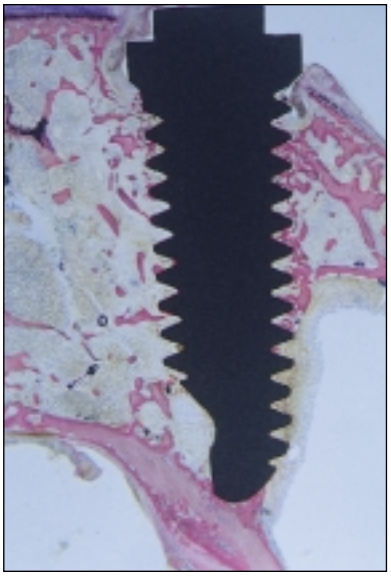

Fig 4 Sandblasted implant at 2 weeks. Many small bone trabeculae are present in close contact with the implant surface (acid fuchsin-toluidine blue; magnification ×20).

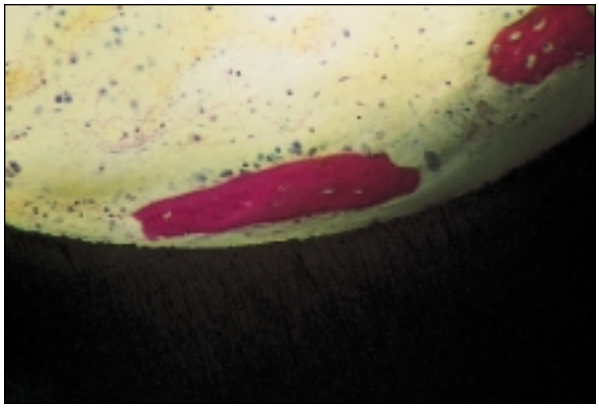

Fig 5 Machined implant at 3 weeks. Osteoblasts are producing bone near the implant surface. A gap is present between the implant and the bone. No osteoblasts are observed directly on the implant surface (acid fuchsin-toluidine blue; magnification ×200).

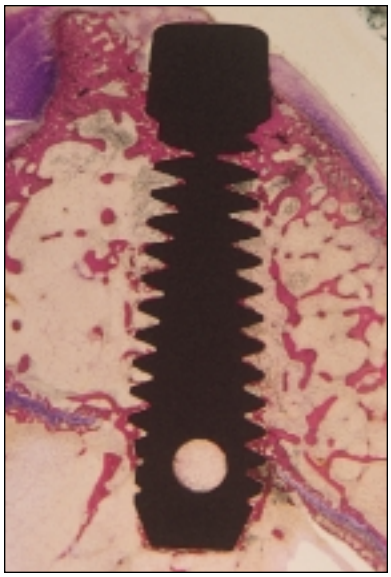

Fig 6 Sandblasted implant at 4 weeks. Small bone trabeculae are present around the major portion of the implant surface (acid fuchsin-toluidine blue; magnification ×20).

Copyright © 2000 by Quintessence Publishing Co, Inc. Printing of this document is restricted to personal use only. No part of this article may be reproduced or transmitted in any form without written permission from the publisher.

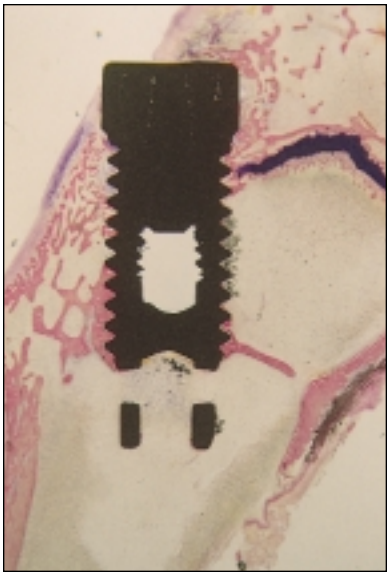

Fig 7 Machined implant at 8 weeks. Few ALP+ osteoblasts are present (acid fuchsin-toluidine blue; magnification ×20).

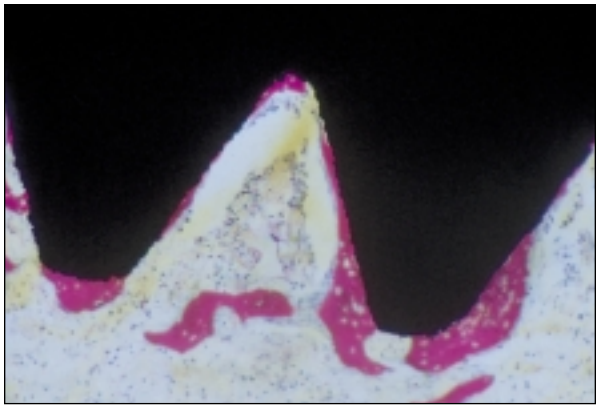

Fig 8 Sandblasted implant at 8 weeks. Small bone trabeculae almost completely surround the implant surface (acid fuchsin-toluidine blue; magnification ×400).

Table 1 Mean Percentages of Direct Implant-Bone Contact in Test and Control Implants at 1 to 8 Weeks After Placement

	Mean percentage of direct bone contact ± SD		
	Test implants	Control implants	Significance of differences
Week 1	5.1 ± 1.1	5.1 ± .9	t = .000; *P* > .1; NS
Week 2	17 ± 4.5	15.1 ± 1.7	t = 1.73; .05 < *P* < .1; NS
Week 3	42 ± 2.8	30 ± 1.8	t = 15.80; *P* < .001; S
Week 4	54 ± 2.6	45 ± 1.6	t = 12.86; *P* < .001; S
Week 8	60 ± 1.4	51 ± 1.9	t = 16.80; *P* < .001; S

S = statistically significant difference; NS = no statistically significant difference; SD = standard deviation.

Sandblasted Implants at 8 Weeks. Mature bone and, only in a few areas, nonmineralized osteoid matrix were present at the interface (Fig 8). Only a few ALP+ osteoblasts were present.

Surface Roughness Measurement. The surface roughness (Ra) was .80 µm on the machined implants, and 2.09 µm on the sandblasted implants.

Statistical Analysis. As expected, in both experimental groups the percentage of direct bone-implant contact showed a statistically significant increase (test implants: F = 1424.1, *P* < .001; control implants: F = 2764.6, *P* < .001) through the study period. A statistically significantly greater amount of bone contact was observed in test implants beginning the third week, as compared to control implants (Table 1).

Discussion

The geometric surface properties of an implant seem to influence the components of the cell cytoskeleton involved in cell spreading and locomotion.[13] Surface roughness can also have an effect on the wettability features of a solid; this wettability seems to have an effect on the configuration and conformation of the proteins deposited on the implant surface, which are important in cell adhesion. Cochran et al[1] found significantly less coronal bone loss for sandblasted and etched (SLA) implants; and this may be the result of the higher osteoconductive properties of the SLA surface. Bowers et al[2] found in their study that the highest quantity of attached cells was found on the rough, irregular sandblasted surfaces.

Future research efforts should be geared toward finding an optimal surface microroughness with an improved understanding of the relationship between the cytoskeletal arrangement of cells and the development of an underlying extracellular matrix and the surface micromorphology.[2] The fact that some cells orient themselves in the grooves of micromachined surfaces supports the concept that cells are sensitive to microtopography.[6] Bowers et al[2] concluded that sandblasted implants provide a unique environment and opportunity for initial cell attachment. Morphometric analysis

Copyright © 2000 by Quintessence Publishing Co, Inc. Printing of this document is restricted to personal use only. No part of this article may be reproduced or transmitted in any form without written permission from the publisher.

has shown a relationship between increased bone-implant contact and surface roughness.[14]

The diameter of blasting particles also seems to be important. Wennerberg et al[15,16] found that the percentage of bone-implant contact was greater when a blasting particle size of 25 µm was used rather than 250 µm; that the surface roughness measurement (Ra) was .82, 1.32, and 2.11 for implants blasted with 25-µm, 75-µm, and 250-µm particles, respectively[3,15,18]; and that a stronger inflammatory response was seen with 250-µm particles. This last fact could be the result of an increasing ionic leakage related to the increased surface roughness.[15,18]

The histomorphometric results showed a significantly higher percentage of bone-implant contact from the third week onwards, and these values are similar to those reported by Gotfredsen et al.[7] These data could be related to the higher surface roughness of the sandblasted implants; their measured roughness values (Ra) were 2.09, versus .80 for the machined control implants. This study confirms, moreover, the data of Wennerberg et al,[3] who found no negative effects in spite of the presence of Al ions on the implant surface following the sandblasting procedure.

A different type of bone growth was found around the machined and the sandblasted implants; in the first group, the bone growth was implantopetal, ie, from the host bed toward the implant surface, while in the second group, the growth appeared to be implantofugal, ie, from the implant toward the host bed.[17]

Conclusion

The bone growth pattern in sandblasted implants, together with their apparently higher osteoconductive surface could explain the significantly higher bone-implant contact percentages observed in this study. More studies are certainly needed, especially removal torque evaluation of implants with different surface morphologies,[18] to find the surface that can offer the best anchorage for dental implants.

Acknowledgments

This work was partially supported by the National Research Council (CNR) and by the Ministry of University, Research, Science, and Technology (MURST).

References

1. Cochran DL, Nummokoski PV, Higginbottom FL, Hermann JS, Makins SR, Buser D. Evaluation of an endosseous titanium implant with a sandblasted and acid-etched surface in the canine mandible: Radiographic results. Clin Oral Implants Res 1996;7:240–252.
2. Bowers KT, Keller J, Randolph BA, Wick DG, Michaels CM. Optimization of surface micromorphology for enhanced osteoblast responses in vitro. Int J Oral Maxillofac Implants 1992;7:302–310.
3. Wennerberg A, Albrektsson T, Lausmaa J. Torque and histomorphometric evaluation of c.p. titanium screws blasted with 25- and 75-µm-sized particles of Al_2O_3. J Biomed Mater Res 1996;30:251–260.
4. Schwartz Z, Martin JY, Dean DD, Simpson J, Cochran DL, Boyan BD. Effect of titanium surface roughness on chondrocyte proliferation, matrix production, and differentiation depends on the state of cell maturation. J Biomed Mater Res 1996;30:145–155.
5. Garvey BT, Bizios R. A transmission electron microscopy examination of the interface between osteoblasts and metal biomaterials. J Biomed Mater Res 1995;29:987–992.
6. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford DJ, et al. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG 63). J Biomed Mater Res 1995;29:389–401.
7. Gotfredsen K, Wennerberg A, Johansson C, Skovgaard LT, Hjørting-Hansen E. Anchorage of TiO_2-blasted, HA-coated and machined implants: An experimental study with rabbits. J Biomed Mater Res 1995;29:1223–1231.
8. Feighan JE, Goldberg VM, Davy D, Parr JA, Stevenson S. The influence of surface-blasting on the incorporation of titanium-alloy implants in a rabbit intramedullary model. J Bone Joint Surg 1995;77-A:1380–1395.
9. Wennerberg A, Albrektsson T, Johansson C, Andersson B. Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography. Biomaterials 1996;17:15–22.
10. Piattelli A, Piattelli M, Scarano A. Simultaneous demonstration of alkaline and acid phosphatase activities in bone, at bone implant interface and at the epiphyseal growth plate in plastic embedded undemineralized tissue. Biomaterials 1997;18:545–549.
11. Piattelli A, Scarano A, Quaranta M. High-precision, cost-effective system for producing thin sections of oral tissues containing dental implants. Biomaterials 1997;18:577–579.
12. Piattelli A, Scarano A, Piattelli M. Detection of alkaline and acid phosphatases around titanium implants: A light microscopical and histochemical study in rabbits. Biomaterials 1995;16:1333–1338.
13. Den Braber ET, De Ruijter JE, Smits HTJ, Ginsel LA, Von Recum AF, Jansen JA. Effect of parallel surface microgrooves and surface energy on cell growth. J Biomed Mater Res 1995;29:511–518.
14. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants: A histomorphometric study in miniature pigs. J Biomed Mater Res 1991;25:889–902.
15. Wennerberg A, Albrektsson T, Andersson B. Bone tissue response to commercially pure titanium implants blasted with fine and coarse particles of aluminum oxide. Int J Oral Maxillofac Implants 1996;11:38–45.
16. Wennerberg A. On surface roughness and implant incorporation [thesis]. Gothenburg: Univ of Gothenburg, 1996.
17. Piattelli A, Scarano A, Piattelli M, Calabrese L. Direct bone formation on sand-blasted titanium implants. An experimental study. Biomaterials 1996;17:1015–1018.
18. Wennerberg A, Albrektsson T, Andersson B, Kroll JJ. A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies. Clin Oral Implants Res 1995;6:24–30.

COPYRIGHT © 2000 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART OF THIS ARTICLE MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.