BONE RESPONSE TO MACHINED AND RESORBABLE BLAST MATERIAL TITANIUM IMPLANTS: AN EXPERIMENTAL STUDY IN RABBITS

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KEY WORDS

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The aim of the present study was a comparison of implants' responses to a machined surface and to a surface sandblasted with hydroxyapatite (HA) particles (resorbable blast material [RBM]). Threaded machined and RBM, grade 3, commercially pure, titanium, screw-shaped implants were used in this study. Twenty-four New Zealand white mature male rabbits were used. The implants were inserted into the articular femoral knee joint according to a previously described technique. Each rabbit received 2 implants, 1 test (RBM) and 1 control (machined). A total of 48 implants (24 control and 24 test) were inserted. The rabbits were anesthetized with intramuscular injections of fluanisone (0.7 mg/kg body weight) and diazepam (1.5 mg/kg b.wt.), and local anesthesia was given using 1 mL of 2% lidocaine/adrenalin solution. Two rabbits died in the postoperative course. Four animals were euthanatized with an overdose of intravenous pentobarbital after 1, 2, 3, and 4 weeks; 6 rabbits were euthanatized after 8 weeks. A total of 44 implants were retrieved. The specimens were processed with the Precise 1 Automated System to obtain thin ground sections. A total of 3 slides were obtained 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, and histomorphometric analysis was performed. With the machined implants, it was possible to observe the presence of bone trabeculae near the implant surface at low magnification. At higher magnification many actively secreting alkaline phosphatase positive (ALP+) osteoblasts were observed. In many areas, a not yet mineralized matrix was present. After 4 to 8 weeks, mature bone appeared in direct contact with the implant surface, but in many areas a not yet mineralized osteoid matrix was interposed between the mineralized bone and implant surface. In the RBM implants, many ALP+ osteoblasts were present and in direct contact with the implant surface. In other areas of the implant perimeter it was possible to observe the formation of an osteoid matrix directly on the implant surface. Mature bone with few marrow spaces was present after 4 to 8 weeks. Beginning in the third week, a statistically significant difference ($P < .001$) was found in the bone-implant contact percentages in machined and RBM implants. It must be stressed that these results have been obtained in a passive, nonloaded situation.

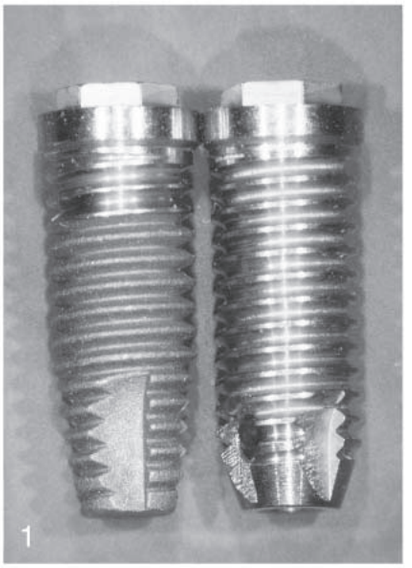
Introduction

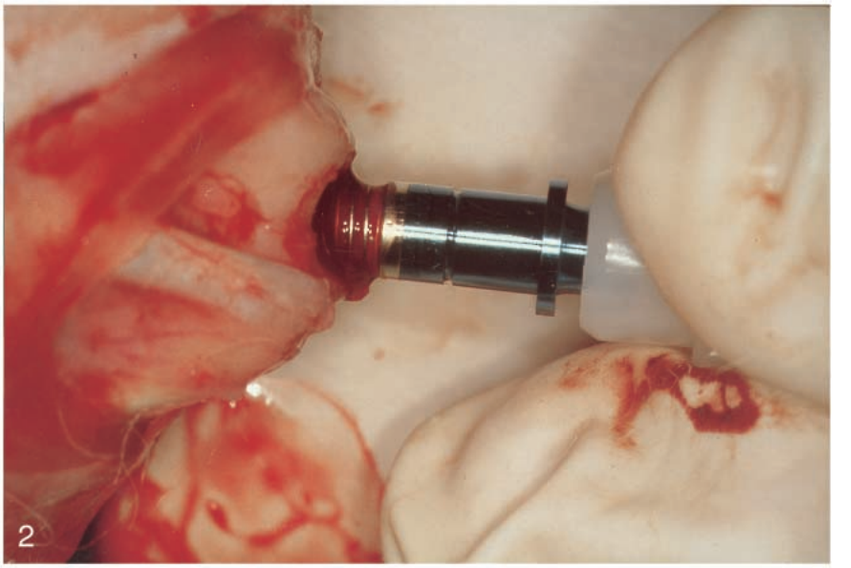
Dental implants' surface morphology, including microgeometry and roughness, has been shown to have a significant effect on implant integration.[1–6] The surfaces of titanium dental implants have been modified by additive methods (titanium plasma spray) or by subtractive methods (acid-etching, sandblasting) to increase the surface area.[6] It has been shown that considerable differences with respect to design and surface topography produced by the surface treatment can be present among commercially prepared implants.[3] A greater surface roughness increases the potential for biomechanical interlocking.[6] The microscopic features of the implant surface have an influence on the cells located at the bone-biomaterials interface.[7–12] In vitro, osteoblasts were affected by the surface roughness in their rate of proliferation and differentiation.[11] Cells cultured on rougher surfaces showed an increased matrix production and an increased alkaline phosphatase expression[2,13] and the characteristics of more differentiated osteoblasts.[2] Osteoblast-like cells were shown to be able to differentiate not only between surfaces of different roughness but also between surfaces of similar roughness but different topography.[13] Not only the surface roughness, but also the ionic charge, surface energy, surface tension, and other still-undefined properties may be of importance in the osseointegration process.[6] The optimal degree and type of surface roughness have not yet been conclusively defined.[6]

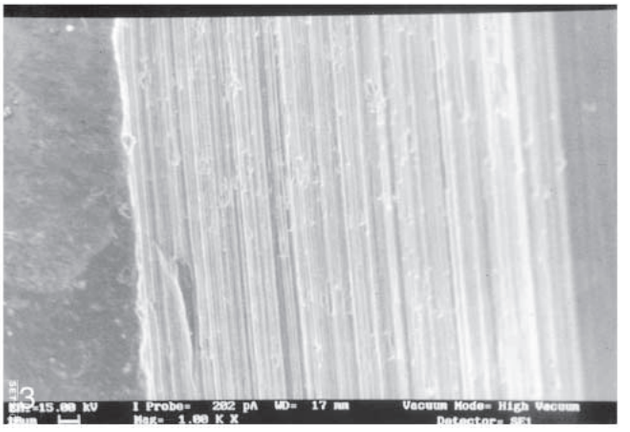
Surface blasting is a process by which metal surfaces are treated with different types of materials (Al_2O_3, TiO_2, etc) to provide an irregular surface.[14,15] Blasted surfaces show a rough, irregular topography.[10] The surface blasting also eliminates the surface contaminants and increases the metal surface reactivity.[16] Some of the blasted implants showed the presence of areas of direct bone apposition on the metal surface, and no space was present between the new bone and the implant surface.[15] Surface blasting can increase the rate and amount of bone formation on the implant surface.[15] Even if no negative effects of Al ions were seen on the peri-implant bone tissues in Al_2O_3-blasted implants,[10] a highly biocompatible material such as hydroxyapatite (HA) has been considered useful as a blasting material. Surface roughness modifications can have a negative influence on the microcomposition, crystallographic structure, and surface energy, and subsequently on the biological response, of the implant. The aim of the present study was a comparison of the bone response to implants with a machined surface and with a surface sandblasted with HA particles.

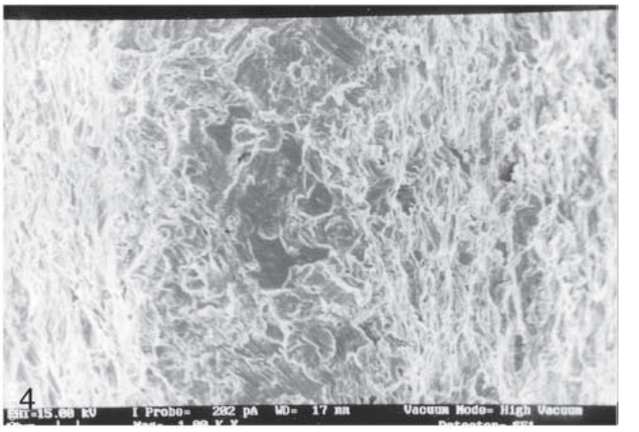
Materials and Methods

Threaded machined and sandblasted with HA particles (resorbable blast material [RBM]), grade 3, commercially pure, titanium, screw-shaped implants (Restore, Lifecore, Chaska, Minn) were used in this study (Figure 1). The implants had been sandblasted with HA (HA crystallinity $> 67\%$, size of HA particulate $-40/+80$ mesh). The implant was then passivated (ASTM F04) to remove any HA particulate and cleaned using the following steps: water rinse, 3 ultrasonic cleaning steps, water rinse, distilled water agitation, alcohol agitation, and air-blown drying. The protocol was approved by the ethics committee of our university. Twenty-four New Zealand white mature male rabbits were used for this study. The implants were inserted into the articular femoral knee joint according to a previously described technique (Figure 2).[17] Each rabbit received 2 implants, 1 test (RBM) and 1 control (machined). A total of 48 implants (24 control and 24 test) were inserted. The rabbits were anesthetized with intramuscular injections of fluanisone (0.7 mg/kg body weight) and diazepam (1.5 mg/kg body weight), and local anesthesia was given using 1 mL of 2% lidocaine/adrenalin solution. A skin incision with a periosteal flap was used to expose the articular surface. The preparation of the bone site was done with burs under generous saline irrigation. The implant insertion was performed by hand. The periosteum and fascia were sutured with catgut and the skin with silk. Two rabbits died in the first 3 days because of postoperative complications; the lost implants were 2 test and 2 controls. Four animals were euthanatized with an overdose of intravenous pentobarbital after 1, 2, 3, and 4 weeks; 6 rabbits were euthanatized after 8 weeks. A total of 44 implants were retrieved. Implants and surrounding tissues were washed in saline solution and immediately fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in a 0.15 molar cacodylate buffer at 4°C and pH 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)[18] and were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate 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 obtained 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.[19] The histomorphometry was done under a Laborlux-S light microscope (Leitz, Wetzlar, Germany) using an Intel Pentium II 300 MMX, a video-acquired schedules Matrox, a video camera, and KS 100 software (Zeiss, Hallbergmoos, Germany). The images acquired were analyzed using the described software system. Three test and 3 control implants were analyzed under a Leo scanning electron microscope









FIGURES 1–4. FIGURE 1. The threaded titanium implants used in this study. On the left-hand side the resorbable blast material (RBM) implant, and on the right-hand side is the machined implant. FIGURE 2. The implant is inserted in the femoral knee joint. FIGURE 3. A machined implant. Typical machining grooves are present on the surface (×1000). FIGURE 4. A resorbable blast material (RBM) implant. The surface is highly irregular with depressions and small diameter peaks (×1000).

(Zeiss, Hallbergmoos, Germany). Roughness measurements were performed for the machined and the sandblasted implants using a Mitutoyo Surftest 211 Profilometer (Mitutoyo Corporation, Tokyo, Japan): an average of 3 readings was performed for each surface. A total of 5 machined and 5 sandblasted implants were analyzed.

RESULTS

Scanning electron microscopy (SEM)

Machined Implants

It was possible to observe on the surface the presence of typical machining grooves produced by the manufacturing instruments (Figure 3).

RBM Implants

The appearance of the implants' surfaces was glazed and it was possible to observe a very rough surface produced by the RBM procedure (Figure 4). The surface was highly irregular with many depressions and small diameter indentations.

Surface Roughness

The surface roughness (Ra) of machined implants was 0.78 μm, whereas that of sandblasted implants was 2.14 μm.

Light microscopy

One Week (Control)

At low magnification it was possible to observe the presence of bone trabeculae near the implant surface (5.1% ± 0.94% bone-implant contact). At higher magnification many actively secreting alkaline phosphatase positive (ALP+) osteoblasts were observed. In many areas, a not yet mineralized matrix was present.

One Week (Test)

Newly formed bone surrounding implant surfaces and many osteoblasts producing an osteoid matrix were observed. Bone-implant contact was similar to the control sites (5.1% ± 1.14%). Many ALP+ osteoblasts were present and in direct contact with the implant surface. In other areas of the implant perimeter it was possible to observe the formation of an osteoid matrix directly on the implant surface.

Two Weeks (Control)

An increase in the number of bone trabeculae was observed. Bone trabeculae were present near the implant surface (15.1% ± 1.74% bone-implant contact; Figure 5). Many ALP+ osteoblasts were present, and they were secreting an osteoid matrix toward the implant surface (ie, in an implantopetal direction).

Two Weeks (Test)

A bone-implant direct contact in RBM implants was observed (Figure 6). A great quantity of newly formed bone was observed in close contact with implant RBM surfaces. Bone-implant contact was 17.1% ± 4.5% and revealed a great percentage of bone osteoconductivity, which was probably due to the HA particles. In some fields the osteoid matrix was undergoing mineralization.

Three Weeks (Control)

A higher quantity of bone (30% ± 1.82% bone-implant contact) and ALP+ osteoblasts around the implants were observed (Figure 7). A 2- to 5-μm gap between the newly formed bone and implant surface was observed.

Three Weeks (Test)

An increase in the number of ALP+ osteoblasts and a bone-implant close contact of 42.45% ± 2.95% were observed. Many osteoblasts were located directly on the implant surface (Figure 8), whereas in other regions an osteoid matrix and bone were present. No gaps were present between the bone and the implant.

Four Weeks (Control)

Mature bone with few marrow spaces was present (45% ± 1.65% bone-implant contact). A sharp decrease in the number of ALP+ osteoblasts was seen. Only in a few areas was bone in direct contact with the implant.

Four Weeks (Test)

The ALP+ osteoblasts decreased; 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. Bone-implant contact was 54.9% ± 2.80%.

Eight Weeks (Control)

The quantity of bone was slightly higher than that observed at 4 weeks (51% ± 1.90% bone-implant contact). Only in a few areas were ALP+ osteoblasts observed. Mature bone appeared in direct contact with the implant surface (Figure 9), but in many areas a not yet mineralized osteoid matrix was interposed between mineralized bone and the implant surface.

Eight Weeks (Test)

Only a few ALP+ osteoblasts were present. Mature bone and a not yet mineralized osteoid matrix (although only in a few areas) were present at the interface (Figure 10). Bone-implant contact was 62.3% ± 4.30%.

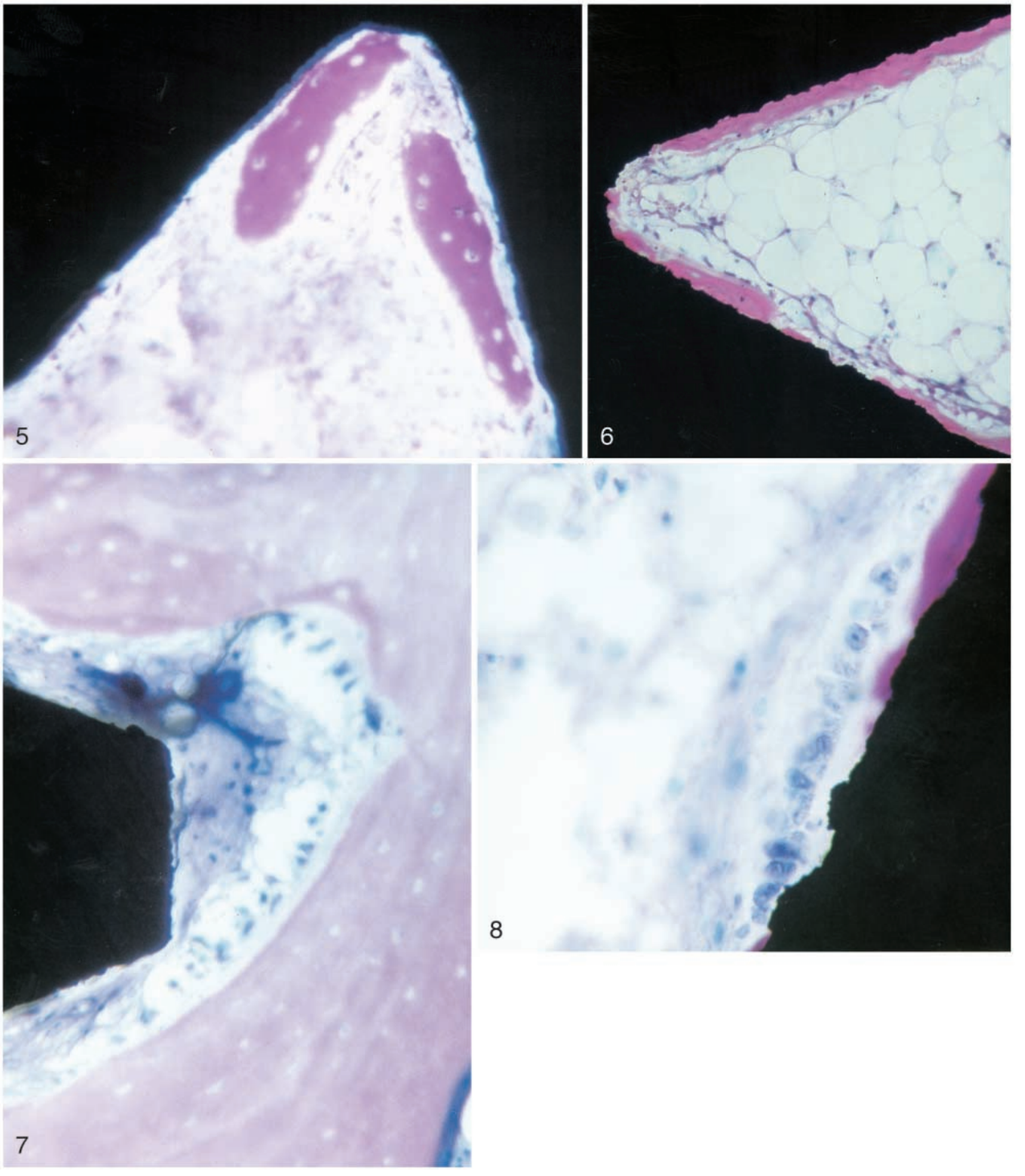
Statistical analysis

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 significant greater amount of bone contact was observed in test implants from the third week as compared with control implants ($P < .001$).

Discussion

The aim of the present study was a comparison of the response in implants with a machined surface and implants with a surface sandblasted with HA particles (RBM). In machined implants, after 4 to 8 weeks mature bone appeared in direct contact with the implant surface, but in many areas a not yet mineralized osteoid matrix was interposed between mineralized bone and the implant surface. In RBM implants, on the contrary, many alkaline phosphatase positive osteoblasts were present and in direct contact with the implant surface. In other areas of the implant perimeter it was possible to observe the formation of an osteoid matrix directly on the implant surface. From the third week a great percentage of bone-implant close contact was present in RBM implants, and this percentage increased after 4 and 8 weeks. In conclusion, a higher bone-implant contact percentage was observed with the sandblasted RBM implants, and this surface could be considered more osteoconductive than a machined one.

The geometric surface properties seem to affect the components of the cell cytoskeleton involved in cell spreading and locomotion.[20] Surface roughness can also have an affect on the wettability features of a solid: this wettability seems to have an affect on the configuration and conformation of the proteins deposited on the surface and are important in cell adhesion. Cochran et al[7] found significantly less coronal bone loss for sandblasted and acid-etched (SLA) implants, and this could be due to the higher osteoconductive properties of the SLA surface. Bowers et al[9] found that the highest quantity of attached cells was found on the rough, irregular, sandblasted surfaces. In the future, research efforts should be aimed at finding an optimal surface microroughness and an improved understanding of the relationship between the cytoskeletal arrangement of the cells and the development of an underlying extracellular matrix and the surface micromorphology.[9] The fact that some cells can orient in the grooves of micromachined surfaces supports the concept that cells are sensitive to microtopography.[13] Bowers et



FIGURES 5–8. FIGURE 5. A machined implant. After 2 weeks it is possible to observe newly formed bone trabeculae that are separated by a small gap from the implant surface (acid fuchsin-toluidine blue ×100). FIGURE 6. A resorbable blast material (RBM) implant. Osteoblasts are deposing an osteoid matrix and bone directly on the implant surface (acid fuchsin-toluidine blue ×100). FIGURE 7. A machined implant. The osteoblasts are producing bone toward the implant surface (acid fuchsin-toluidine blue ×100). FIGURE 8. A resorbable blast material (RBM) implant. A rim of osteoblasts is deposing an osteoid matrix and bone directly on the implant surface (acid fuchsin-toluidine blue ×200).

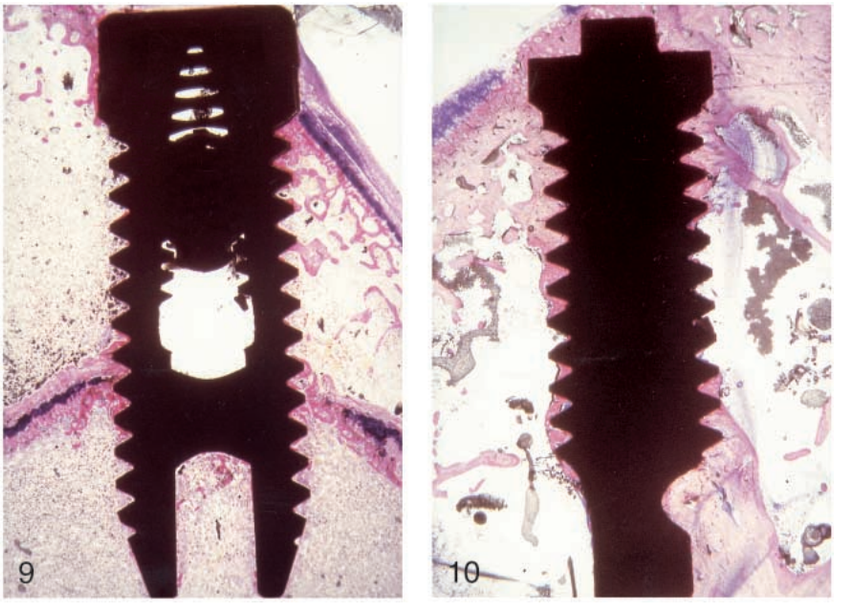


FIGURE 9. A machined implant. Mature bone is present around the implant (acid fuchsin-toluidine blue ×12). FIGURE 10. A resorbable blast material (RBM) implant. A high bone-implant contact percentage is present (acid fuchsin-toluidine blue ×12).

al[9] concluded that sandblasted implants provide a unique environment and opportunity for initial cell attachment. The morphometric analysis showed a relationship between the increase in bone-implant contact and the surface roughness.[21] The diameter of blasting particles also seems important. Wennerberg et al[21–24] found that the percentage of bone-implant contact was greater for 25 μm than 250 μm; that the surface roughness measurement (Ra) was 0.82, 1.32, and 2.11 for implants blasted with 25, 75, and 250 μm, respectively[10,22,24]; and that a stronger inflammatory response was seen with the latter. This fact could be due to an increasing ionic leakage related to the increased surface roughness.[22,24] Our histomorphometric results showed a significantly higher percentage of bone-implant contact from the third week onward, and these values are similar to those reported by Gotfredsen et al.[14]

These data could be related to the higher surface roughness measurements of the RBM-sandblasted implants: the values (Ra) were 2.14 vs 0.78 μm for the machined control implants. A different type of bone growth was found around the machined and the RBM implants: in the first group, the bone growth was implantopetal (ie, from the host bed toward the implant surface), whereas in the second group the growth appeared to be implantofugal (ie, from the implant toward the host bed).[8,25] This fact could explain, together with the apparently higher osteoconductive properties of an RBM surface, the significantly higher bone-implant contact percentages observed in our study. More studies are certainly needed, especially regarding the removal torque evaluations of implants with different surface morphologies,[24] in order to try to find the surface that could offer the best anchorage for dental implants. It must also be stressed that the different type of bone growth (implantofugal vs implantopetal) and the higher bone-implant contact percentage found around RBM implants could be especially useful in exacting clinical conditions like poor quality bone and early or immediate loading.

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