Sandblasted and Acid-etched Dental Implants: A Histologic Study in Rats

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Purpose: *Current literature has revealed that surface etching of endosseous implants can improve bone-implant contact. The aim of this study was to evaluate the differences in bone-implant contact (BIC) between sandblasted/acid-etched and machined-surface implants.* ***Materials and Methods:*** *Thirty-two Sprague-Dawley rats were used in this study. Two implant surfaces, Ecotek (sandblasted/acid-etched) and machined, were used with 1 implant placed in each tibia of the animals. A total of 64 implants were placed. BIC was evaluated at 5, 15, 30, and 60 days. Histomorphometry of the BIC was evaluated statistically.* ***Results:*** *The sandblasted/acid-etched surface demonstrated a greater BIC percentage than the machined surface. This difference was statistically significant only at 30 and 60 days after healing.* ***Discussion and Conclusion:*** *The sandblasted/acid-etched surface demonstrated a stronger bone response than the machined one at a later period of healing.* (Int J Oral Maxillofac Implants 2003;18:75–81)

Key words: *dental acid etching, dental implants, osseointegration, surface properties*

The bone regeneration around dental implants is a function of various types of implant surfaces.[1–3] The roughness of the surface can be achieved with subtractive methods (sandblasting, acid etching) or additive methods (titanium plasma spraying, hydroxyapatite [HA] coating).[4,5] The sandblasted surface is achieved by treatment of the surface with a spray of air and abrasives for a certain period of time and under controlled pressure. Various materials are used as abrasives, for example, aluminum oxide (Al_2O_3), with particles measuring from 25 to 250 µm.[6,7] The diameter of the used particles seems to be an important factor. Wennerberg and coworkers[8] reported that the percentage of bone-implant contact (BIC) was greater at the surface blasted with particles of 25 µm in comparison to that blasted with the 250-µm particles, and also that the inflammatory response was greater in the implants treated with particles of greater diameter.[8,9] This fact could be the result of increased ionic dispersion correlated with the excessive increase of the surface roughness.

To avoid a possible risk that the presence of aluminum ions on the surface of the implant could inhibit normal bone mineralization, particles of titanium dioxide (TiO_2) have been used as sandblasting material.[10] With this method, the surface of the implant is treated without the use of foreign elements. A recent technique of sandblasting using particles of HA has been described (RBM; Resorbable Blast Material).[11] This technique has the main objective of creating a rough surface; and if any HA particle should be left on the surface, no problems would arise, because HA is highly biocompatible and possibly osteogenic.

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The acid etching technique has been used with the goal of avoiding the disadvantages of the sandblasted surface, ie, the contamination of the titanium by the materials used in the blasting, the nonhomogeneous treatment of the surface, and the risk of loss of the metallic material, which could reduce the mechanical resistance of the implant.[12–14] The etching can be achieved by treating of the surface with hydrochloric acid and sulfuric acid (HCl/H_2SO_4) or a mixture of hydrofluoric acid and nitric acid (HF 2%/HNO_3 10%).

A study in rabbits compared the removal torque values for an acid-etched surface and a machined surface, and the results were statistically superior for the implants with the etched surface.[15] To obtain a sandblasted and etched surface in a single phase of treatment, the surface is blasted with materials of greater diameter that results in a macrorough surface; this is followed by a bath in an acid solution that produces microirregularities and increases the implant surface area.[16–19] This second phase is accomplished by the acid solution of hydrochloric/sulfuric acid or hydrofluoric/nitric acid. Even though it has been reported in the literature that the Al_2O_3 used in the blasting procedure is biocompatible and does not interfere with the osseointegration process, the etching phase could help to remove contaminants of the surface and increase reactivity of the metal.[20–22]

In a study by Buser and coworkers in minipigs,[23] the removal torque of sandblasted/acid-etched (SLA [sandblasted, large-grit, acid-etched]) implants and machined/acid-etched implants was compared. It was found that the removal torque of the SLA implants was significantly greater. In the same study, scanning electron microscopy (SEM) of the surface revealed a plane profile for the etched surface. This was later confirmed by profilometric analyses, which indicated greater roughness for the SLA surface (Ra = 2.0 μm) in comparison with the etched surface (Ra = 1.3 μm).[24–27]

The aim of this study was to analyze the bone healing around machined and SLA implants placed in the rat tibia.

MATERIALS AND METHODS

SLA and machined (M) mini implants (2.0×2.0 mm) (Bone System, Milano, Italy) were used in this study. The surface of the SLA implant used in this study (Ecotek, Bone System) was first treated with medium granules of ruby corundum (250 to 500 μm) and then with HF solution at 1% and HNO_3 at 30%. The average surface roughness (Ra) of the machined implants was 0.86 μm, while that of the SLA implants was 2.15 μm. A total of 32 Sprague-Dawley young male rats were used in this study. The rats were divided into 2 groups. The test group received the SLA implants, while the control group received the M implants. After the rats were anesthetized by an intraperitoneal injection of 8% chloral hydrate (400 mg/100 mg body weight), both legs were shaved and washed. Surgery was performed under sterile conditions. The medial aspect of the proximal tibial metaphysis was exposed through an anteromedial skin incision. With a series of drill guides and burs, 1 implant was placed in each tibia. The sterilized implants were placed bilaterally into the surgically prepared cavities by manual tapping. Profuse irrigation with sterilized physiologic saline solution was maintained throughout the entire procedure. The skin was closed in layers with absorbable sutures. After the surgery, the animals were housed with free access to water and food. No antibiotics were given.

Histologic Procedures

The animals were sacrificed at 5, 15, 30, and 60 days after implant placement. Four animals per group were sacrificed at each time point. They were anesthetized in the same manner as mentioned above. The proximal section of the tibia with the implants was removed en bloc and immediately stored in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).[28] The specimens 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 longitudinally along the major axis of the implant with a high-precision diamond disc at about 150 μm and ground down to about 30 μm. Three slides were obtained for each implant, and samples were stained with basic fuchsin and toluidine blue. Histomorphometry of BIC percentage was done under a Laborlux-S light microscope (Leitz, Wetzlar, Germany) using an Intel Pentium III 300 MMX, a video-acquired schedules Matrox, a videocamera, and KS 300 Software (Zeiss, Hallbergmoos, Germany). The images acquired were analyzed using the described software system.

Data Analysis

The differences in the percentage of bone contact between test (SLA) and control (M) implants were evaluated. The BIC percentages were expressed as the means ± standard deviation and standard error. The differences between the 2 groups were analyzed by analysis of variance (ANOVA), and statistical

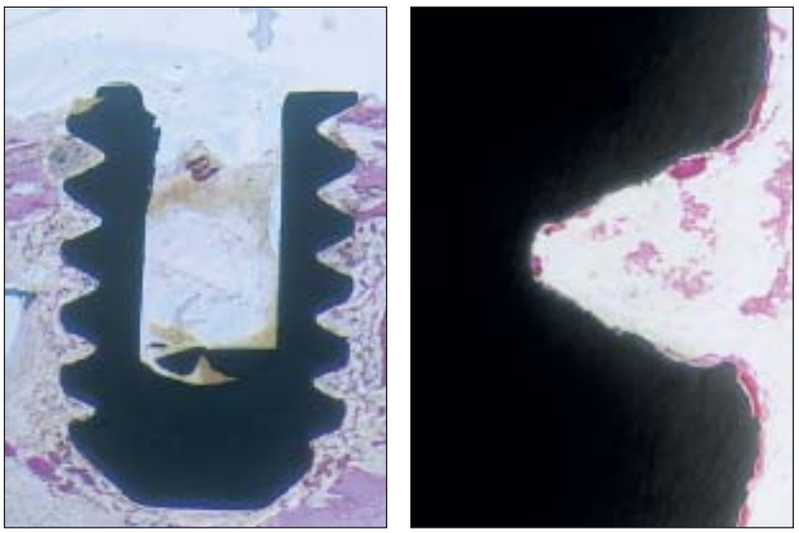

Fig 1a *(Left)* M implant at 15 days. Little bone formation can be seen (original magnification ×12).

Fig 1b *(Right)* Higher magnification. Little osteogenic activity is evident, and only a small amount of new bone is in contact with the titanium surface (original magnification ×100).

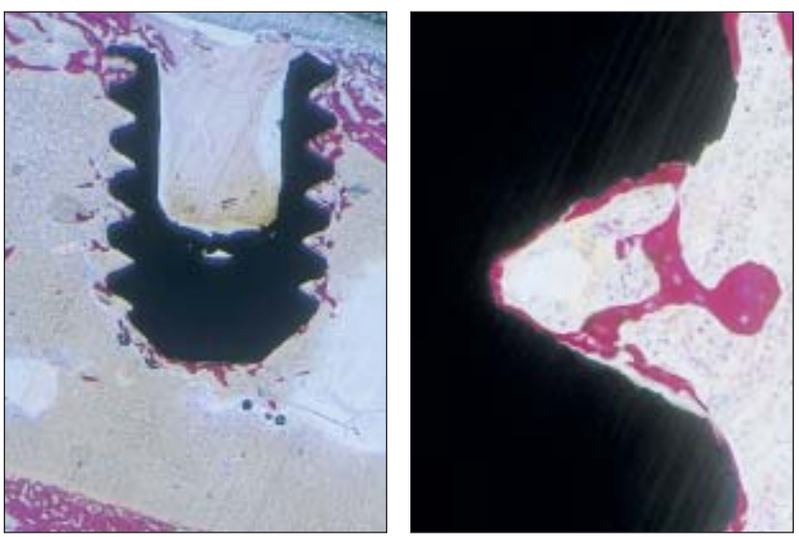

Fig 2a *(Left)* SLA implant at 15 days. New trabeculae can be seen around the implant, and new bone formation is evident toward the apex (original magnification ×12).

Fig 2b *(Right)* Higher magnification of the SLA surface. New bone formation can be seen in intimate contact with the threads. There is no inflammatory infiltrate at the interface level. It is possible to observe immature osteoblasts near the implant surface (original magnification ×100).

significance of multiple comparisons was evaluated using the Fisher PLSD and Scheffe F tests. Significance was set at $P \leq .05$.

RESULTS

Implants at 5 Days

In the M implants, newly formed bone could be seen deposited by osteoblasts near the implant surface. In this surface, neither osteoclasts nor multinucleated giant cells were observed. The mean BIC percentage was 9.66 ± 1.5. In the SLA implants, newly formed bone trabeculae in direct contact with the implant surface and adjacent areas could be seen. Multinucleated cells and osteoclastic cells were not observed. The mean BIC percentage was 10 ± 1.0.

Implants at 15 Days

In the M implants, trabecular bone formation could be seen in contact with the implant surface; numerous osteoblasts secreting osteoid matrix could be observed toward the implant. Fewer bone trabeculae were seen around the implant surface. New bone formation could be seen in this surface, but low osteoblastic activity was present around the implant surface. The mean BIC percentage was 13.3 ± 2.0 (Figs 1a and 1b).

In the SLA implants, the formation of bone trabeculae occurred directly in contact with the implant surface; many osteoblasts secreting osteoid matrix were observed on the implant surface. A higher number of bone trabeculae were observed adjacent to the implants, in comparison with the M implants. The mean BIC percentage was 12.0 ± 1.5 (Figs 2a and 2b).

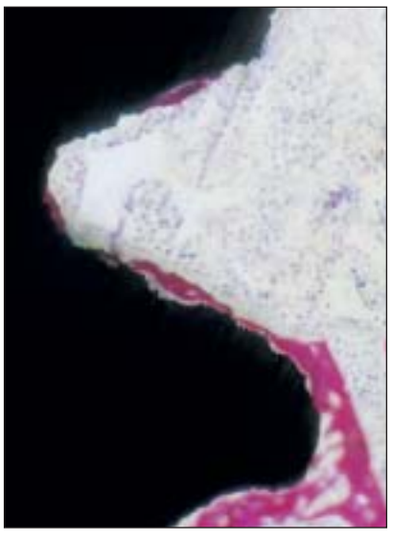

Fig 3a *(Left)* M implants at 30 days. There is a minimal increase in the bone-implant contact (original magnification ×12).

Fig 3b *(Right)* Higher magnification. There is a minimal increase in the trabeculation of the newly formed bone (original magnification ×100).

Fig 4a *(Left)* SLA surface at 30 days. New bone is evident, with bone trabeculae around the implant surface. Medullary spaces are present, indicating good vascularization of the newly formed bone. Trabecular bone is more evident at the bone interface (original magnification ×12).

Fig 4b *(Below)* Higher magnification. New bone formation is more visible at the bone-implant interface. There is osteoblastic activity, and no inflammatory cells are present at the interface (original magnification ×100).

Implants at 30 Days

In the M implants, little mature bone contact was seen on the implant surface. The mean BIC percentage was 42.0 ± 4.5 (Figs 3a and 3b). In the SLA implants, mature mineralized bone was observed in the cortical and medullary region. The latter presented marrow spaces and the bone was more mature. The mean BIC percentage was 54.0 ± 2.0 (Figs 4a and 4b).

Implants at 60 Days

Around the M implants, mature compact bone with no gaps at the bone-implant interface was present. Some marrow spaces were present. The mean BIC percentage was 53.0 ± 1.4 (Figs 5a and 5b). Around the SLA implants, mature bone with small marrow spaces was observed. Small bone trabeculae in the marrow spaces were present. No connective tissue was present at the bone-implant interface. The mean BIC percentage was 60.6 ± 1.5 (Figs 6a and 6b).

Statistical Analysis

At 5 and 15 days, statistical analysis of the difference between the 2 surfaces revealed no significant differences (P = .1593). At 30 days and 60 days, the difference between the 2 surfaces was statistically significant (P = .041 and P = .0014, respectively) (Table 1).

Fig 5a *(Left)* M surface at 60 days. More mature bone is contacting the threads (original magnification ×12).

Fig 5b *(Right)* Higher magnification. The machined surface shows a gap between the mature bone and the titanium surface, but no connective tissue is present (original magnification ×100).

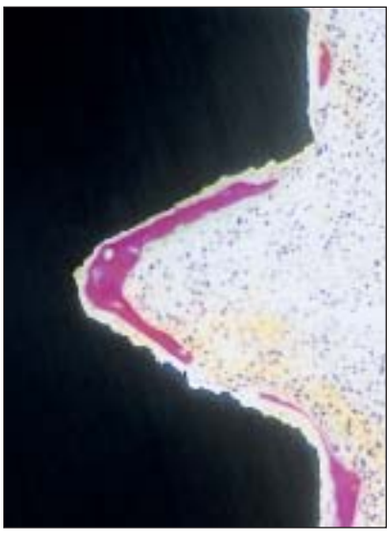

Fig 6a SLA surface at 60 days. There is mature bone with small marrow spaces around the SLA surface. No connective tissue is present between the bone and the implant. Lamellar bone is in contact with the implant surface (original magnification ×12).

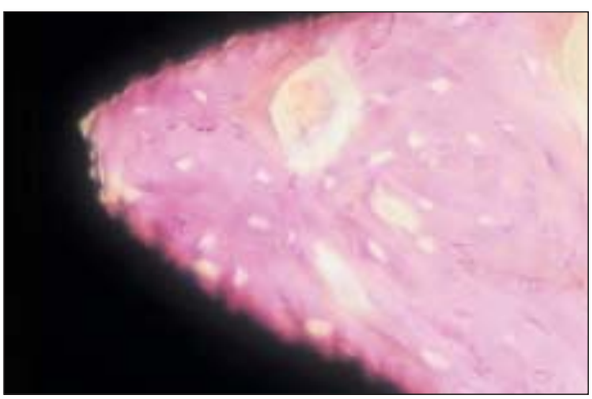

Fig 6b Higher magnification. It is possible to observe new compact bone with few medullary spaces. There are no gaps at the interface level and there is no fibrous connective tissue (original magnification ×100).

Table 1 Statistical Comparison of Bone-Implant Contact Percentage (BIC%) at Sandblasted/Acid-etched (SLA) and Machined (M) Surfaces

	BIC%			
Time	**Mean**	**SD**	**SE**	***P* value**
5 days				
SLA	10.0	1.0		.1593†
M	9.66	1.527		
15 days				
SLA	12.0	1.527		.1593†
M	13.33	2.081		
30 days				
SLA	54.0	2.0	1.15	.041*
M	42.0	4.58	2.65	
60 days				
SLA	60.66	1.528	0.882	.0014*
M	53.0	1.41	0.707	

SD = standard deviation; SE = standard error. †Non-significant; *Significant at 95% (according to the Fisher PLSD and Scheffe F test).

DISCUSSION

An increase in the implant surface area seems to enhance biomechanical bone-implant bonding.[15] Irregular implant surfaces can influence bone cells adjacent to the implant, increasing their proliferation and differentiation. Cells cultured on rougher surfaces have demonstrated an enhancement of bone matrix and an expression of alkaline phosphatase. The production of other factors involved in bone growth, for example, osteocalcin, is also greater on rougher surfaces. These factors seem to increase the regeneration potential at the bone-implant interface, improving the bone integration of implants. Macrophages appear to prefer rough surfaces instead of the machined ones, while fibroblasts tend to prefer machined surfaces.[29–34]

The effects that the superficial irregular layer can have on the formation of different growth factors by the cells has not yet been clarified, but the fact that the chemical composition of titanium surfaces is identical makes it probable that different superficial topography can modulate cell behavior.[35] Torque removal is the necessary force to break the union between bone and implant in removing the implant itself. It can be used to measure the anchorage provided by bone-implant contact of osseointegration: the greater the force needed to remove the implant, the greater the percentage of BIC.

Several studies have confirmed that removal torque is significantly greater for irregular surfaces than machined surfaces.[8,10,18] There is also experimental evidence that among irregular surfaces, some of them demonstrate superior removal torque to others. In a histomorphometric study by Wennerberg and coworkers, the percentage of direct bone contact at machined and sandblasted implants was compared.[8] They found that the percentage for the latter was 62%, compared to 50% for the machined surface. In the same study, the percentages of direct bone contact were significantly greater for the sandblasted implant with 25-μm particles (Ra = 1.16 μm) compared to the sandblasted implant with 250-μm particles (with Ra = 1.94 μm). There was also a positive correlation between direct bone contact and the torque removal of the implant. The sandblasting process creates superficial roughness, and the acidification process creates additional deep roughness. The roughness can increase the adhesion of the osteoblast-like cells and seems to have an effect on the configuration and conformation of cellular pseudopodia, which are important in cell adhesion. Bowers and associates[5] evaluated the response of the osteoblasts derived from the rat calvaria on different titanium surfaces. A significant level of cellular adhesion was observed on the rough surfaces, especially those with irregular morphology. It was also concluded that the sandblasted implants presented a particular opportunity for the initial cellular attachment. Moreover, it seems that sandblasting followed by an acid etching procedure can increase the roughness of the implant surface, positively influencing the adhesion and proliferation of the cells. The fact that some cells could be guided into the sulcus of the smooth surface, as reported by Martin and coworkers,[24] supports the concept that they are sensitive to the microtopography of the surface. In fact, the geometric properties of the surface have an influence on the cytoskeleton components that are responsible for growth, movement, and cell adhesion. It is well documented in the aforementioned literature that the rough surfaces stimulate cell proliferation and differentiation, thereby increasing production of chemical mediators and growth factors.

CONCLUSIONS

The results of this study showed that the test (SLA) surface was demonstrated to have a greater osteogenic activity than the control (M) surface; this was statistically significant only at 30 and 60 days after healing. However, more studies need to be done to find ways to promote BIC during the initial phases of healing.

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