Histologic Evaluation of Early Human Bone Response to Different Implant Surfaces

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Background: Studies have demonstrated that roughened dental implant surfaces show firmer bone fixation and an increased percentage of bone-to-implant contact (BIC%) compared to commercially pure titanium-surface (machined) implants. Therefore, the purpose of this study was to evaluate the influence of implant-surface topography on human bone tissue after 2 months of unloaded healing.

Methods: Fourteen subjects with a mean age of 46.87 ± 9.45 years received two microimplants each (2.5 mm in diameter and 6 mm in length), one test (sandblasted acid-etched surface) and one control (machined surface), either in the mandible or in the maxilla. After a healing period of 2 months, the microimplants and surrounding tissues were removed with a trephine bur and prepared for histologic analysis.

Results: All microimplants, except for one of the controls, were clinically stable after the healing period. Histometric evaluation indicated that the mean BIC% was 23.08% ± 11.95% and 42.83% ± 9.80% for machined and rough microimplant surfaces, respectively ($P = 0.0005$). The bone area within the threads was also higher for sandblasted-surface implants ($P = 0.0005$). The mean percentage of bone density did not differ between the two groups ($P = 0.578$).

Conclusion: Data from the present histological study suggest that the sandblasted acid-etched implant provides a better human bone tissue response than machined implants under unloaded conditions after a healing period of 2 months. *J Periodontol 2006;77:1736-1743.*

KEY WORDS

Dental implants; histology; osseointegration; titanium; wound healing.

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Several investigators have demonstrated higher removal torque values and percentage of bone-to-implant contact (BIC%) for rough dental implant surfaces compared to machined surfaces.[1-3] These studies have also shown that dental implants inserted in type IV bone (posterior maxilla and grafted areas) may result in a higher ratio of early failures than implants placed in dense bone. However, most of the studies have used commercially pure titanium (machined) dental implants, and it is possible that a modification of the dental implant surface can facilitate healing and increase BIC% in areas with soft bone tissue.[4-6] This background triggered the search for implant-surface modifications using techniques such as grit blasting, titanium plasma spraying, acid etching, anodic oxidation, and laser preparation.[7-9] The dental implant quality depends on the chemical, physical, mechanical, and topographic properties of the surface. These different properties interact and determine the activity of the cells close to the dental implant surface.[10]

The sandblasted acid-etched surface is obtained by treating the commercially pure titanium dental implant with a spray of air and abrasive material (aluminum oxide or titanium oxide) for a certain period of time and under controlled pressure. Next, this modified surface is attacked with acid solutions under different

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temperatures and periods of time to remove any residue and to condition the blasted surface. In addition, the properties of this surface influence cell migration and proliferation, resulting in better BIC%.[7-12]

Few studies and case reports have been published evaluating the peri-implant bone response in humans to different implant surfaces.[13-17] Therefore, the quality of the bone-implant interface around sandblasted acid-etched surfaces after a short period of healing is still to be determined.

The objective of this study was to evaluate the influence of different implant topographies on bone-to-implant contact after an unloaded healing period of 2 months in human jaws.

MATERIALS AND METHODS

Subject Selection

Fourteen partially edentulous subjects (eight females and six males) with a mean age of 46.87 ± 9.45 years, who were referred to the Department of Periodontology, Guarulhos University, for oral rehabilitation with dental implants in the posterior region between August 2004 and April 2005, were included in the present study. Exclusion criteria included pregnancy, nursing, smoking, and any systemic condition that could affect bone healing. The Ethical Committee for Human Clinical Trials approved the study protocol.

Implant-Surface Topographies

In this study, screw-shaped microimplants made of grade-4 titanium§ were prepared with two surface morphologies: machined and sandblasted acid-etched surfaces. Each microimplant was 2.5 mm in diameter and 6.0 mm in length.

The acid-etch process (HCl/HNO_3) was controlled to create a homogeneous implant-surface topography. The dental implants were blasted with 25 to 100 μm TiO_2 particles. After sandblasting, the dental implants were ultrasonically cleaned with an alkaline solution, washed in distilled water, and bathed with a mixture of HNO_3 and HF.

An optical laser profilometer‖ was used to measure and characterize the dental implant-surface topography. Six microimplants from both groups (three microimplants from each group) were measured three times each on the side, top, and bottom. The measured parameters, such as the arithmetic average of all profile-point absolute values (Ra), the root mean square of all point values (Rq), and the average absolute-height values of the five highest peaks and depths of the five deepest valleys (Rz) were measured in all specimens of both groups.

Experimental Design

Twenty-eight screw-shaped microimplants were used in this study. Fourteen machined microimplants served as controls and 14 sandblasted acid-etched surfaces served as tests (Fig. 1). Each subject received two microimplants, one test and one control, which were inserted in the posterior region of the mandible or maxilla, always distal to the last conventional implant placed.

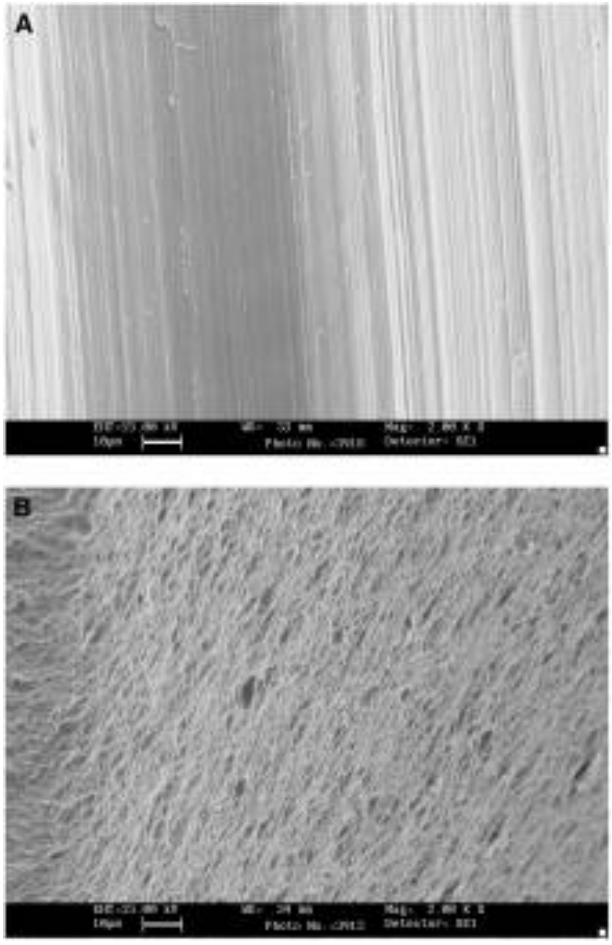

Figure 1.
*Scanning electron microphotograph of the microimplant surface topographies evaluated: machined **(A)** and sandblasted acid-etched **(B)** surface.*

Surgical Procedures

Implants were placed under aseptic conditions after a crestal incision and the elevation of mucoperiosteal flaps. The surgical sites were prepared either with a 1.8-mm-diameter twist drill in the maxilla or a 2.0-mm-diameter twist drill in the mandible. Afterwards, the microimplants were inserted with a screwdriver. If the microimplant presented low primary stability, a second surgical site was prepared. Drilling procedures and microimplant placements were made under profuse irrigation with sterile saline. Flaps were sutured with single interrupted sutures, submerging the

§ Conexão Dental Implants, São Paulo, SP, Brazil.
‖ Mahr, Gottingen, Germany.

microimplants. A total of 300 mg clindamycin was given three times a day for 1 week to avoid postsurgical infection. A total of 50 mg of diclofenac potassium was administered for pain control three times a day for 5 days. Sutures were removed after 10 days. For postoperative dental-biofilm control, subjects were prescribed 0.12% chlorhexidine rinses twice a day during 14 days.

After a healing period of 2 months, the microimplants and surrounding tissues were retrieved with a 4.0-mm-wide trephine bur, and the specimens were fixed by immediate immersion in 4% neutral formalin.

Processing of Specimens and Histometric Analysis

Biopsies were processed to obtain thin ground sections[¶] as previously described.[18] Specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycol methacrylate resin.[#] After polymerization, specimens were sectioned longitudinally along the major axis of the implant with a high-precision diamond disk at ~150 μm and ground down to ~30 μm. Three slides were obtained for each implant. The slides were stained with basic fuchsin and toluidine blue. BIC% was measured around all implant surfaces. The bone density in the thread area (BA%) and the bone density (BD%) in a 500-μm-wide zone lateral to the implant surface were measured bilaterally. These evaluations were performed using a light microscope[**] connected to a high-resolution video camera[††] and interfaced to a monitor and personal computer. This optical system was associated with a digitizing pad and a histometry software package with image-capturing capabilities.[‡‡]

The mean and standard deviation of histometric-variable values were calculated for each implant and then for each group. The Wilcoxon matched-pairs signed-rank test was used to evaluate differences between the implant surfaces. The Mann-Whitney U test was used to evaluate the differences between the implant placed in the different jaws (maxilla or mandible). The level of significance was set at 5%.

RESULTS

Surface Roughness

Table 1 shows the profilometry measurements. The sandblasted acid-etched surface showed a higher mean value for all parameters ($P<0.05$). In addition, the surface topography of the machined surface was well defined, whereas the sandblasted acid-etched–surface topography had no clear orientation.

Clinical Observations

Six subjects received implants in the posterior maxilla, and eight subjects in the posterior mandible. All microimplants, except for one with a machined surface placed in the maxilla, were found to be clinically stable at the time of retrieval. The unstable microimplant was excluded from the histometric evaluation.

None of the microimplants presented marginal bone resorption or a surrounding infection. The test group (sandblasted acid-etched surface) presented more bone attached to the surface than the control group (machined surface) (Fig. 2).

Table 1.

Mean ± SD of Machined and Sandblasted Acid-Etched–Surface Profilometry

Implant Surface Topography*	Ra (μm)	Rq (μm)	Rz (μm)
Machined	0.32 ± 0.03	0.43 ± 0.02	4.20 ± 3.00
Sandblasted acid-etched surface	0.73 ± 0.04	0.95 ± 0.06	5.67 ± 0.26

Ra = the arithmetic average of absolute values of all profile points; Rq = the root mean square of values of all points; Rz = the average value of absolute heights of the five highest peaks and depths of the five deepest valleys.
* Statistically significant between implant surface topographies (Mann-Whitney U test; $P<0.05$).

Figure 2.
Detail of retrieved microimplants from the maxilla. Note the difference in bone-surface density between the machined (left) and sandblasted acid-etched–surface (right) implants.

Histologic and Histometric Results

The bone tissue surrounding all microimplants was healthy (Fig. 3). The old bone was mostly lamellar and compact, and numerous osteocytes were observed in the lacunae, although areas of woven bone could be distinguished. The newly formed bone

¶ Precise 1 Automated System, Assing, Rome, Italy.
# Technovit 7200 VLC, Kulzer, Wehrheim, Germany.
** Laborlux S, Leitz, Wetzlar, Germany.
†† 3CCD, JVC KY-F55B, Milan, Italy.
‡‡ Image-Pro Plus 4.5, Media Cybernetics, Silver Spring, MD and Immagini & Computer Snc, Milan, Italy.

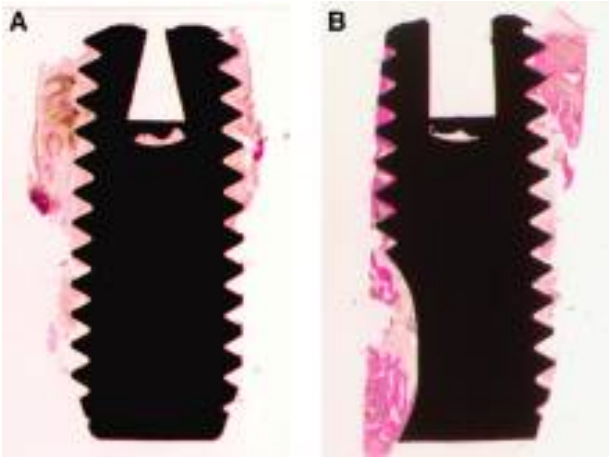

Figure 3.
Histologic ground section of the microimplants presented in Figure 2. ***A)*** *The machined surface depicted newly formed bone, although there is a lack of connecting bridges between new bone trabeculae and the machined surface.* ***B)*** *Ground section of the sandblasted surface presenting newly formed bone which exhibited early stages of maturation and remodeling. (Basic fuchsin and toluidine blue staining; original magnification: A and B, ×12.)*

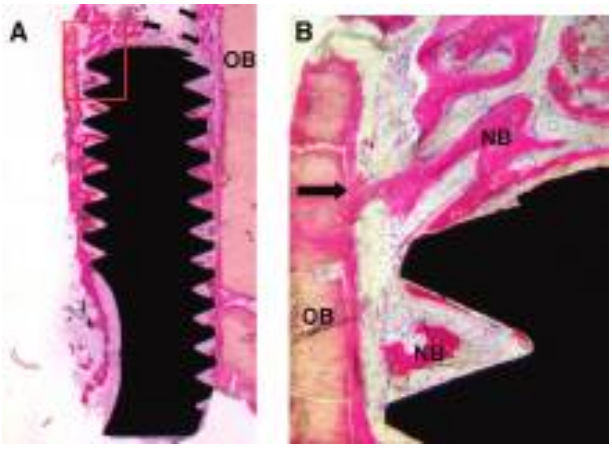

Figure 4.
Histologic ground section of a microimplant with sandblasted surface retrieved from the mandible. ***A)*** *The old bone (OB) was mostly lamellar and compact, and numerous osteocytes were present in the lacunae, although areas of new bone could be distinguished (arrows).* ***B)*** *Magnification of boxed area in A. There is a connecting bridge between the old bone (OB) and the thin new bone (NB) as indicated by the arrow. A minor apposition of new bone is depicted in close contact with the implant surface. (Basic fuchsin and toluidine blue staining; original magnification: A, ×12; B, ×200.)*

exhibited early stages of maturation and remodeling, mainly in the test group (Fig. 4).

In some cases, osteoblasts were connected to the newly formed bone, indicating ongoing bone formation. Inside the implant threads, a minor apposition of new bone could be found, although the bone tissue appeared immature (Fig. 5). Some samples depicted

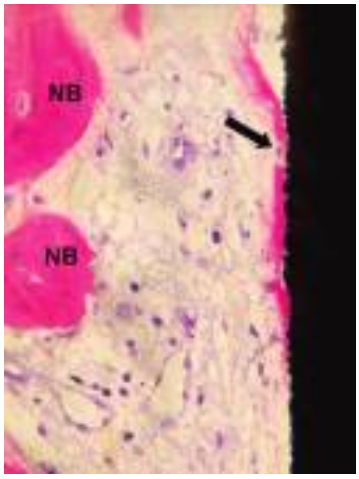

Figure 5.
A thin layer of bone tissue with osteocyte in direct contact with sandblasted surface (arrow) suggesting osteogenesis (basic fuchsin and toluidine blue staining; original magnification ×200).

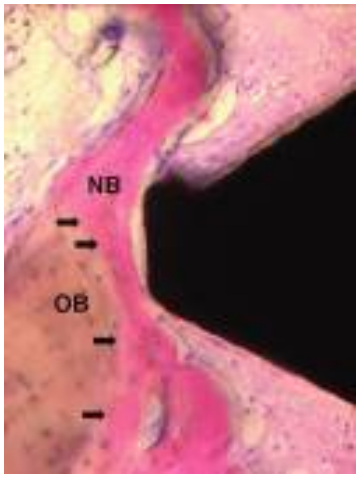

Figure 6.
Reversal lines (arrows) showing the limits between old bone (OB) and new bone (NB). Note that the area close to the machined surfaces shows a thin layer of dense connective tissue between the bone and the machined surface (basic fuchsin and toluidine blue staining; original magnification ×200).

a lack of connecting bridges between the thin bone trabeculae and the machined-implant surface. On the sandblasted acid-etched–surface group, a thin layer of bone trabeculae was interposed between the old bone and implants. The area close to the machined surfaces showed a thin layer of dense connective tissue between the bone and implants (Fig. 6).

None of the test microimplants presented surface debris or particle inclusions in the surrounding tissue close to the bone area. In addition, some sections displayed inflammatory cells (lymphocytes, macrophages, and giant cells) near the implant surface,

mainly in the sandblasted acid-etched implants. However, this inflammatory reaction did not alter the bone-healing response around sandblasted acid-etched–implant surfaces.

The sandblasted acid-etched–surface implants presented significantly higher means of BIC% and BA% (42.83% and 56.93%) compared to the control group (23.08% and 31.40%; Fig. 7). The mean BD% did not differ between tests (27.00%) and controls (18.99%). Figures 8 and 9 present the histometric results according to the location of the microimplants in the arch: maxilla or mandible. The sandblasted-surface implants showed higher mean percentages of BIC and BA independent of the arch analyzed. The mean BD% did not differ significantly between the two groups.

When each group was compared according to their position in the jaw (maxilla or mandible), data showed that both groups presented higher means of BIC% in the mandible (Fig. 10). None of the groups showed significant differences in the mean BA% between the maxilla and mandible. Interestingly, the BD% for machined surfaces presented higher means in the mandible ($P = 0.015$), whereas the sandblasted surfaces presented no differences between the maxilla and the mandible ($P = 0.150$).

DISCUSSION

In the present study, the sandblasted acid-etched–surface implants exhibited a considerable percentage of mineralized bone contact compared to machined-surface implants. The modified surface topography presents a geometric property that functions as a mechanical restriction for cytoskeletal cell components, which are involved in spreading and locomotion.[19,20] Additionally, it has been suggested that acid treatment enhances early bone-implant integration to a level similar to that observed around a more complex surface topography, such as titanium plasma-sprayed and hydroxyapatite-coated surfaces.[20-22]

All clinically stable microimplants showed histologic new bone formation near both implant surfaces. A thin layer of bone covered a relatively large portion of the microimplant threads with sandblasted acid-etched surfaces. These data suggest that osteoblasts were activated by the sandblasted acid-etched surface, suggesting a direct contact osteogenesis reported by other authors.[23] However, woven bone

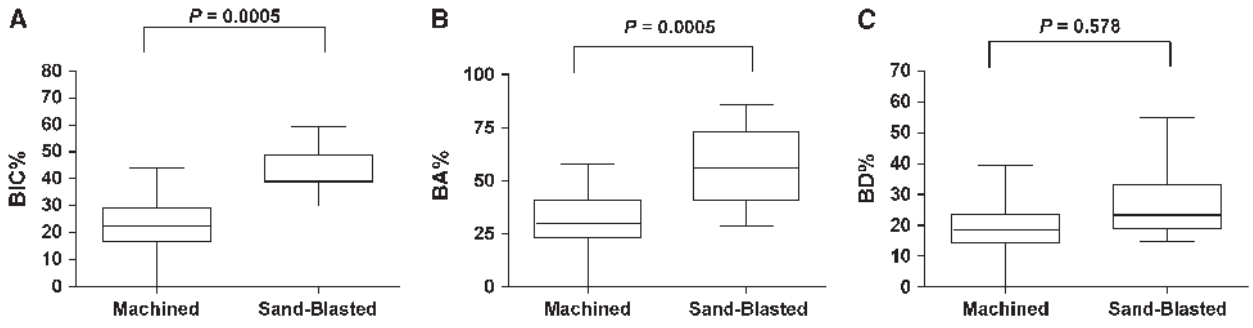

Figure 7.
*Box plots of BIC% **(A)**, BA% **(B)**, and BD% **(C)** in a 500-μm-wide zone lateral to the microimplant for machined and sandblasted acid-etched surfaces in the maxilla and mandible (N = 13 subjects) (Wilcoxon matched-pairs signed-rank test; P <0.05).*

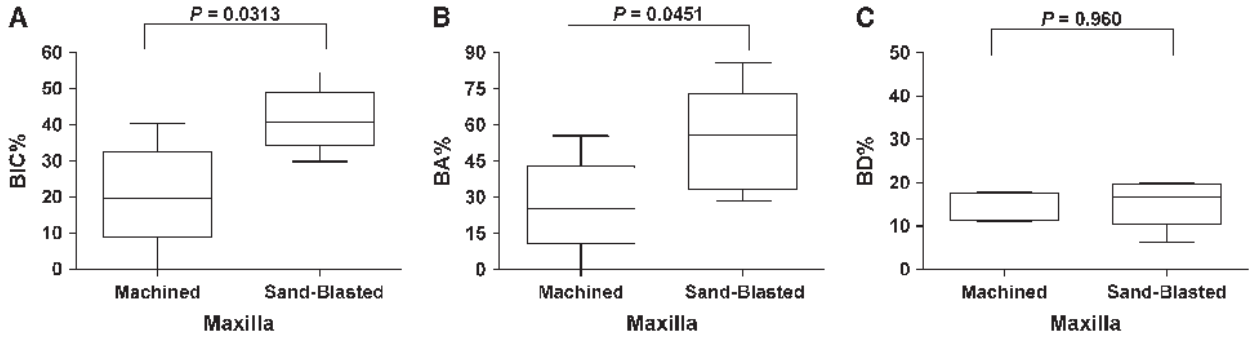

Figure 8.
*Box plots of BIC% **(A)**, BA% **(B)**, and BD% **(C)** in a 500-μm-wide zone lateral to the microimplant for machined and sandblasted acid-etched surfaces in the maxilla (N = 5 subjects) (Wilcoxon matched-pairs signed-rank test; P <0.05).*

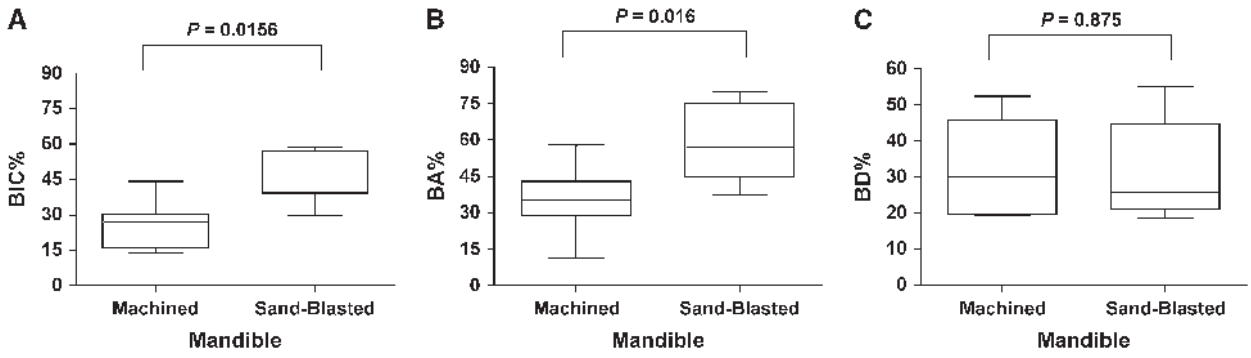

Figure 9.
*Box plots of BIC% **(A)**, BA% **(B)**, and BD% **(C)** in a 500-μm-wide zone lateral to the microimplant for machined and sandblasted acid-etched surfaces in the mandible (N = 8 subjects) (Wilcoxon matched-pairs signed-rank test; P <0.05).*

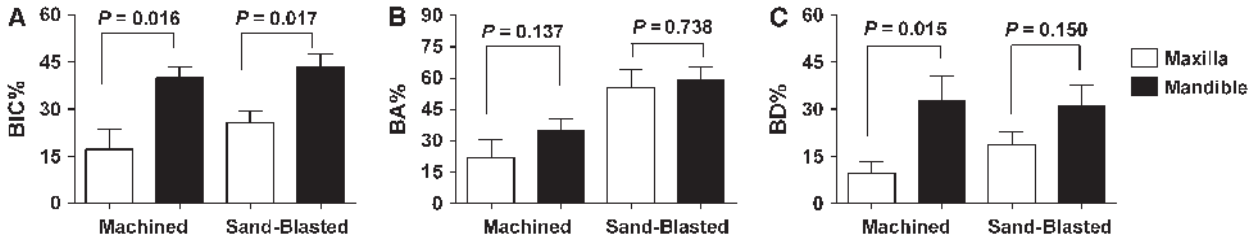

Figure 10.
*Comparison (mean and SD) of BIC% **(A)**, BA% **(B)**, and BD% **(C)** in a 500-μm-wide zone lateral to the microimplant for machined and sandblasted acid-etched surfaces in the maxilla and mandible (Mann-Whitney U test; P <0.05).*

was also found on both surfaces evaluated at some distance from the old bone. This feature is called distant osteogenesis.[23] Complementarily, some histologic slides also depicted osteoblasts lining the newly formed bone, although this characteristic was less pronounced in the machined-surface implants. Macrophages and other inflammatory cells were visible in regions with close soft-tissue implant contact; however, this histological feature had no adverse effect on bone-to-implant contact to sandblasted acid-etched surfaces.

The data from the present experimental study suggest that the surface topography is important for the bone-tissue response to a dental implant, mainly during unloaded conditions.[2,14,15,21,24] The proliferation and differentiation of bone cells have been reported to be enhanced by roughness of the implant-surface topography.[25,26] The healing is initiated immediately after implant insertion by initial blood-clot formation in the peri-implant gaps and the development of a layer of fibrins.[23-30] In this study, implants with a modified surface showed higher amounts of BIC% and BA%. An important feature was that the bone density in a 500-μm-wide zone lateral to the implant surface around the sandblasted acid-etched implants did not differ between the maxilla and the mandible, suggesting that this surface topography may enhance the bone quality close to dental implants placed in soft bone. In addition, a series of coordinated events, including protein adsorption, proliferation, and deposition of bone tissue were probably affected by different topography surfaces. The stylus profilometry of the oxidized surface could provide a better condition for coagulum stability, facilitating bone healing on the implant surface.

More recently, studies have shown that the implant surface topography itself can affect not only osteoblast gene expression but also differentiation of cells into osteoblasts.[31,32] These authors also suggested that the interaction of the cells with the extracellular matrix components and the organization of the actin cytoskeleton associated with implant surface topography can influence cell gene expression.

Data obtained from the machined dental implant surface agree with the statement that this surface does not provide a strong implant anchorage in bone, mainly in sites with poor bone density such as in the posterior maxilla. These observations could explain the increased failure rates previously reported in several investigations.[4,5,33,34] So far, the surface topography may be one of the most important factors in determining long-term implant survival in type IV bone. The placement of dental implants in type IV bone, in particular in the posterior maxilla, has a lower

success rate than in areas with a better bone quality.[4,6] The histometric results of the present investigation suggest that the use of implants with rough surfaces can enhance the osseointegration process, in agreement with previous studies performed in both animal[2,8,20,21,24,30,35-41] and human bone tissue.[13-16,42]

On the other hand, some studies have demonstrated that the anchorage of machined dental implants is time dependent.[36,37] However, some previous studies in humans[14,15] that evaluated osseointegration on machined surfaces inserted in human jaws showed that the percentage of BIC ranged between 9% and 13% after a 5- to 6-month healing period. These values were lower when compared to the present results and those presented by Trisi et al.[13] and Shibli et al.,[42] suggesting that the machined surface depends on the preexisting bone quality at the implant site more than the surface properties.

CONCLUSION

Data from the present histological study suggest that sandblasted acid-etched implants provide a better human bone tissue response than machined implants under unloaded conditions and after a healing period of 2 months.

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