Relationship Between the Surface Energy and the Histologic Results of Different Titanium Surfaces

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Abstract: The aim of this study was to evaluate, through in vitro and in vivo studies, the existence of a relationship between surface energy, for wettability, and the clinical behavior of dental implants with different surfaces, one with a surface treated by sandblasting with titanium oxide microparticles followed by acid-etching treatment (experimental group) and another with a machined surface (control group). For the in vitro tests, a total of 30 titanium disks (15 disks for each group) were evaluated by scanning electron microscopy and dispersive energy spectroscopy and for surface roughness and wettability. For the in vivo tests, a total of 24 implants (12 implants for each group) were inserted in the tibiae of 6 rabbits and were removed after 30 and 60 days for histologic analysis. The results showed that the implants with the experimental surface presented a low wettability, and it also resulted in highly stimulated new bone formation in vivo, when compared with the control group dental implant. As for the bone formation, differences between the different surfaces seemed evident, both in quantity and in quality, as implants from the experimental group showed a higher new bone deposition than that from the control group. Thus, in vitro and in vivo tests demonstrated an excellent biologic response of the surfaces treated by sandblasting with microparticles of titanium oxide followed by acid etching.

Key Words: Dental implants, sandblasting with titanium oxide microparticles, wettability, surface treatment, osseointegration

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The physical and chemical characteristics of titanium are relevant and suitable for biomedical applications. In particular, most of its intrinsic properties, such as biocompatibility, low specific weight, high strength-weight ratio, low elasticity modulus, and excellent corrosion resistance, are favorable for dental implant manufacturing.[1] Titanium surface can be easily modified, either by adding a coating consisting of different types of bioactive substances, by removing portions of the external layer with the use of blasting materials of different particle sizes, or by the application of chemical treatments and/or by physical means such as the laser.[2] Among these, blasting and acid etching have been the most widely used by the industry, and their combination has shown improved biologic activity of the titanium surface for implant osseointegration.[3]

The modification of the implant surface could bring benefits to the response of the peri-implant bone tissue, accelerating the healing process and/or improving the newly formed bone quality.[4,5] Several studies have shown that osseointegration is related to microgeometric features, such as the degree of surface roughness, but it could also depend on factors such as physical and chemical surface properties. The latter may increase the surface wettability, enhance cell adhesion, and promote cell proliferation, increasing the bone-to-implant contact area.[2,6] On the other hand, macrogeometric features such as the design of the implant, its height, the number, the step, and the cutting ability of the threads may also affect the biomechanics of the implant-bone interlocking, possibly improving implant stability.[7–10]

Many different types of chemical and physical surface treatments have been developed, and dental implants with different surface treatments have been commercialized by several manufacturers, even if there is still no consensus on what would be the best characteristics to assure an optimal peri-implant bone growth. It is known that the bone tissue response can be influenced by the implant surface topography at the micrometric level, and some indication exists that a nanometric surface can also have an effect.[11,12] However, the mechanisms behind an optimal bone response in relation to a given type of surface still remain largely unknown. Some biologic processes involved in the activation of the early stages of osseointegration, such as protein adsorption, cell-surface interaction, progenitor cell recruitment and differentiation, and tissue formation at the interface between the body and the biomaterial, can be affected by the implant surface micro-roughness as well as by its physical-chemical surface properties.[13–15]

Studies on the surface properties using photoelectric spectrometry, scanning electron microscopy, and other techniques have been already described.[16] When changing the implant micromorphology, in vitro tests showed that the surface energy was modified too, so that, in vivo, cell attraction and cellular activity could be changed, thereby modifying the tissue response.

A sandblasted and acid-etched surface was produced by sandblasting with large-grit corundum particles that lead to a macroroughness on the titanium surface.[17,18] This procedure was followed by a strong acid-etching bath with a mixture of HCl/H_2SO_4 at elevated temperatures for several minutes. This produced the fine 2- to 4-μm micropits superimposed on the rough-blasted surface. This type of surface is one of the most documented implant surfaces among those commercially available, demonstrating excellent properties for bone tissue regeneration in different clinical applications and in patients with different conditions.[2,3,13,19]

The aim of this study was to evaluate, through in vitro and in vivo studies, the existence of a relationship between surface energy, for wettability, and the clinical behavior of dental implants with

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Fig 1 4/C

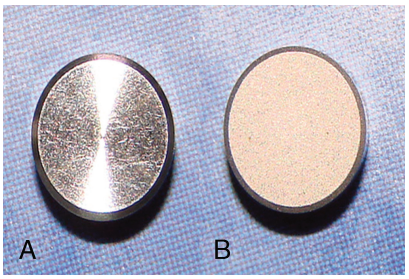

FIGURE 1. Photographs of the disks used in the study, CG (A) and EG (B).

different surfaces, one with a surface treated by sandblasting with titanium oxide microparticles followed by acid-etching treatment [experimental group (EG)] and the other with a machined surface [control group (CG)].

MATERIALS AND METHODS

Preparation of Titanium Disks and Implant Samples

AQ2 Thirty C.P. titanium (titanium grade 4) disks with a 5-mm diameter and 2-mm thickness were fabricated from the same bars used F1 to manufacture titanium dental implants (Fig. 1); 15 had a machined surface (CG), and 15 presented a treated surface (EG). The EG disks were treated by sandblasting with titanium oxide microparticles (size, ~180 μm) followed by a chemical treatment by maleic acid (Implacil DeBortoli, São Paulo/SP, Brazil).

Each sample was prepared, packaged, and sterilized with the same requirements and care of the implant packing. Five disks from each group were used for scanning electron microscope (SEM) and for energy dispersive spectroscopy (EDS); 5 disks from each group, for the assessment of surface wettability; and another 5 from each group, for the roughness surface test.

Twenty-four cylindrical self-tapping implants with internal hexagon, packaged and ready for commercialization, were used for the in vivo study (Implacil DeBortoli, São Paulo/SP, Brazil), 12 machined (CG) and 12 with a treated surface (EG). The implant F2 size was 4 mm in diameter and 8 mm in length (Fig. 2).

In Vitro Characterization of the Chemical-Physical Titanium Surface Characteristics

The evaluation of the chemical-physical titanium surface characteristics was conducted at the Department of Microscopy and Microanalysis and at the Department of Dental Materials of

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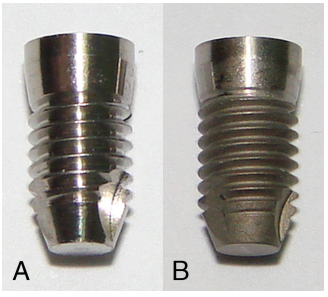

FIGURE 2. Photographs of the dental implants used in this study, the one with the machined surface (A) and the other with the surface treated by sandblasting with titanium oxide microparticles followed by acid-etching treatment (B).

Fig 3 4/C

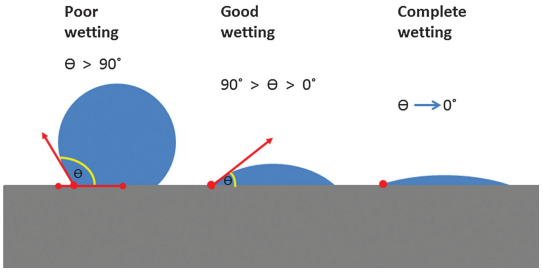

FIGURE 3. Representative image of the surface tension and contact angle wettability.

Pontificia Universidade Católica do Rio Grande do Sul (Porto Alegre/RS, Brazil). The SEM (model JSM 5310; Jeol, Tokyo, Japan) module was used in secondary electron mode, and images were obtained at ×5000 magnification to describe surface topography. The surface composition was evaluated by EDS (model JSM 5310; Jeol, Tokyo, Japan). To analyze the mean surface roughness, that is, the arithmetic mean of the absolute values of the collected roughness data points (Ra), a surface rugosimeter (SJ-201P blend; Mitutoyo, Tokyo, Japan) was used. Finally, another test was designed to check the flow rate of 5 μl of distilled water, applied with a micropipette on the sample. The surface wettability was estimated by measuring the total surface area wetted immediately after the drip. The surface tension was calculated by measuring the contact angle formed between the drop and the disk surface (Fig. 3). For these evaluations, F3 images were taken at different times: 0, 15, 30, and 60 seconds with a high-resolution camera (Sony Cyber-shot DSC-H9; Tokyo, Japan). The images were analyzed using the program ImageTool version 5.02 for Microsoft Windows™ (The University of Texas Health Science Center, San Antonio, TX).

In Vivo Study

This study was approved by the ethics committee of the Federal University of Santa Maria, Rio Grande do Sul, Brazil (protocol number 133/2011). Six adult New Zealand rabbits (*Oryctolagus cuniculus*), weighing approximately 3.5 kg, were used in this study. Two implants were inserted into the proximal metaphysis of each tibia, one of each group, according to a standardized surgical protocol. The implant sites were prepared using proper drills according to the manufacturer's instructions, to a depth of 8 mm, and the implants were manually inserted using the ratchet.

General anesthesia in rabbits was induced by intramuscular injection of ketamine (35 mg/kg; Agener Pharmaceutica, Brazil). Then, a muscle relaxant (Rompum 5 mg/kg; Bayer, Brazil) and a tranquilizer (Acepran 0.75 mg/kg; Univet, Brazil) were injected intramuscularly. In addition, 1 mL of local anesthetic (3% of prilocaine-felypressin; Astra, Mexico) was injected subcutaneously at the site of surgery to improve analgesia and for bleeding control. Postoperatively, a single dose of 600,000 IU of Benzetacil was used. After

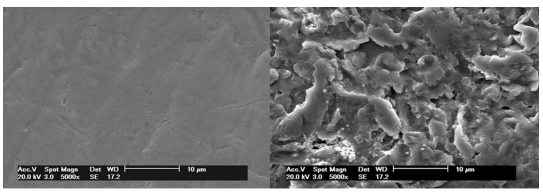

FIGURE 4. The SEM analysis of the CG (A) and EG (B) surfaces at ×5000 magnification.

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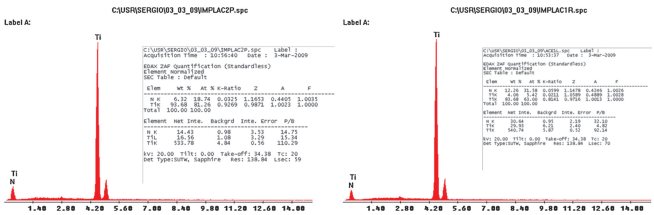

FIGURE 5. The EDS analysis of the CG (A) and EG (B) surfaces.

surgery, the animals were placed in individual cages with 12-hour cycles of light, controlled temperature (21°C) and ad libitum diet, without any particular differences from the diet normally adopted by the laboratory. At the established times, all animals were euthanized with an intravenous overdose of ketamine (2 mL) and xylazine (1 mL). Three animals were euthanized after a period of 30 days; and the other 3, after 60 days. Bone block biopsies were obtained for each implant comprehending surrounding bone.

Histomorphologic Analysis

Bone blocks of the tibiae, with inserted implants, were removed from each animal, placed for fixation in 10% of formaldehyde solution for 7 days, and dehydrated in increasing ethanol solutions (60%, 70%, 80%, and 99%; 24–56 h), as previously described.[20] Then, samples were embedded in Technovit 7200 VLC resin (Kultzer & Co, Wehrhein, Germany) and, after curing, sectioned with a metallographical cutter (Isomet 1000; Buehler, Germany), as previously described.[16] Then, disk samples were polished with an abrasive paper sequence (Metaserv 3000; Buehler, Germany) to ~30 μm of thickness and analyzed by light microscopy (Nikon E200; Japan). The osteocyte counting was made in those sections where the first and third threads that resulted were fully inserted into the bone tissue. The program used for the counting was the Image Pro 4.0 for Windows.

RESULTS

In Vitro Characterization of the Chemical-Physical Titanium Surface Characteristics

The SEM images of the CG showed a surface with smooth grooves caused by the cutting tool during machining, even if most of the analyzed area had a flat surface (Fig. 4A). In the EG, a topographical uniformity may be largely observed, with the presence of deep grooves and a regular microrough surface. Moreover, the edges

F4

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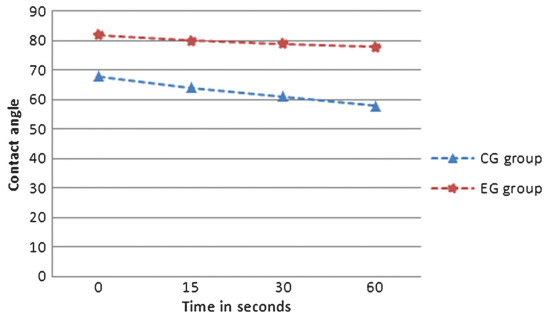

FIGURE 6. Graphic representation of the different behavior in the decreasing of the contact angle values in the 2 groups.

Fig 7 4/C

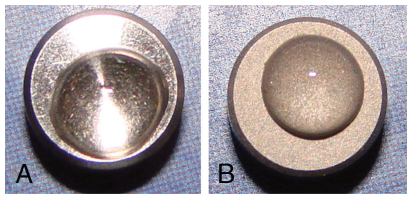

FIGURE 7. Photographs demonstrating the liquid contact on the titanium disk surface.

seemed well rounded due to acid conditioning the surface was subjected to (Fig. 4B). However, a few residues of the blasting particles could be observed at the high-magnification images.

The EDS evaluation showed, in the both groups, a surface with high concentration of titanium (Fig. 5), whereas the presence of other metal ions was not identified. F5

As for the analysis of surface roughness, mean (SD) Ra values of 0.159 (0.033) and 0.699 (0.056) μm were found in disks from the CG and the EG, respectively.

The mean values of the contact angle over different times of water drop were also evaluated (Fig. 6). In the CG, the contact angle values had a continuously, almost linear, decrease at different times, whereas in the EG, the contact angle virtually did not change, remaining stable. As showed in Figure 7, the wetting area resulted higher (27.2%) in the CG after 60 seconds. F6 F7

Histomorphologic Analysis of In Vivo Samples

In the EG, after 30 days, intense areas of new bone formation were visible close to the implant surface, where signs of bone tissue reorganization in the form of lamellar bone were evident. Large blood vessels were also observed in these areas. On the other hand, in the implant from CG, a small cellular response could be seen (Fig. 8). F8

The osteocyte counting recorded in the samples after 30 days had mean values of 34% higher in the EG than in the CG samples.

Samples from the EG taken after 60 days showed better organization, with the osteons occupying most of the area around the implant body, including the spaces between the coils, and allowing a great stimulation of the bone tissue healing around the implant. Another interesting feature shown by these samples was the organization of lamellar bone, which was well distributed at this healing time. On the contrary, samples from the CG showed a good bone formation with a small presence of cells and blood vessels (Fig. 9). F9

Fig 8 4/C

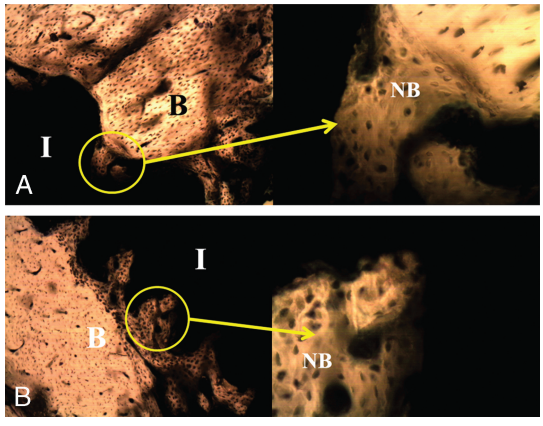

FIGURE 8. Histologic pictures showing difference in the new bone tissue formation, cellular reaction, and lamellar organization in the CG (A) and EG (B) after 30 days in vivo. I, implant; B, bone; NB, newly formed bone tissue.

Fig 9 4/C

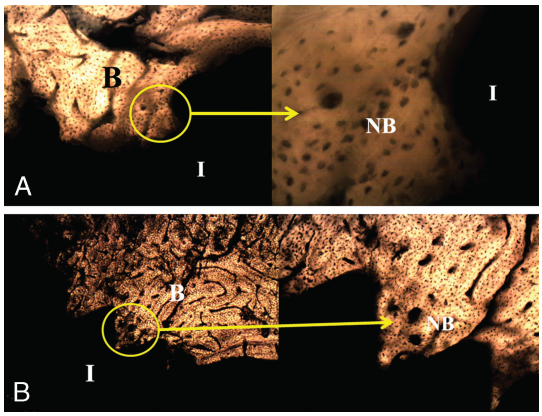

FIGURE 9. Histologic pictures showing difference in the new bone tissue formation, cellular reaction, and lamellar organization in the CG (A) and EG (B) after 60 days in vivo. I, implant; B, bone; NB, newly formed bone tissue.

The osteocyte counting recorded in the samples after 60 days had mean values of 29% higher in the EG than in the CG samples.

DISCUSSION

In recent decades, a series of in vivo studies examined the effect of the implant surface on bone healing and apposition.[21,22] Changes in morphology and surface roughness were initially developed with the aim of increasing the mechanical interlocking between the bone and implant surface, thus improving the initial stability, its resistance, and force dissipation.[23,24]

Histologic studies showed that surface texturing created by blasting led to greater bone-to-implant contact as compared with machined surfaces.[18] Other studies reported that the etching treatment reduced the concentrations of C, Ti, and N on the implant surface but increased the amount of oxygen,[22] revealing a more oxidized surface than the machined one.[25] In this study, EDS evaluation showed, in the EG, a surface with a high concentration of titanium, although only a few microparticles were retained on the surface after blasting. Therefore, the use of these 2 modes of surface treatment produced very interesting and appropriate topographic features. To evaluate the contact angle, it was necessary to consider its relationship with surface chemistry and geometry, that is, the physical and chemical properties of the surface, which were responsible for wettability.[26]

Thermodynamics of surfaces, which considered the minimization of free energy of the system, imposed a single value for the contact angle. However, the wettability test showed that a drop of liquid on the solid titanium surface could be rather stable over time, that is, there was a slight variation in the angle of contact between the 2 samples analyzed. This variation could be seen from the mean values reported in this study, where we could observe that the reduction of the contact angle was linear and progressive in the different times evaluated, demonstrating a greater reduction in machined implants. The titanium surface having received a preliminary blasting with titanium oxide particles promoted in-depth defects, which further may be responsible for the increase in the bone-to-implant contact percentage. This uniform pattern of the surface could be important for surface wetting and cell adhesion.

There was a tendency to assume that the contact angle decreased with the decreasing of the surface tension of the liquid. Still, some authors[26] reported the existence of a positive relationship between the wettability and the surface roughness, expressed as Ra values, that is, the wettability increased with increasing Ra values. These results showed that, after surface conditioning, the implant physical properties could change. In vitro response of cells and tissues were affected by topography, geometry, and macroscopic/microscopic characteristics. Having reduced the contact angle, the interaction of cells with the surface was increased, thus resulting in a probable faster osseointegration.[26] According to the results obtained in this study, the greatest influence on the biologic effects and cell interaction could be attributed more to the surface topographic features than to wettability. In the current study, the good surface properties of the treated implants were confirmed by the in vivo histologic evaluation comparing the groups, with a higher percentage of cells present within of the coils in the treated group compared with controls at both experimental times, 30 and 60 days, demonstrating a difference between the samples in the stimulation of bone healing.

Large blood vessels were observed in these areas; however, this could be considered as a normal finding for the healing time analyzed.

On the basis of these results, it was possible to conclude the following:

The surface roughness of the EG showed a very regular pattern, with peaks and valleys produced by blasting and microporosity obtained by chemical treatment by acids.

The minimal decrease of the contact angle between the water drop and the titanium surface within the periods studied, recorded between the experimental group and the CG, suggested that the wettability was inversely proportional to the quantity and quality of in vivo bone formation.

Histologic analysis showed that the treated surface seemed to promote a more intense bone growth, both qualitatively and quantitatively, and to ensure a better organization of bone tissue compared with the machined implants.

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