The aim of this study was to evaluate the physico-chemical properties and the in vivo host response of a surface sandblasted with particles of titanium oxide (TiO2) followed by acid etching as an alternative to aluminium oxide. Thirty titanium disks manufactured in the same conditions as the implants and 24 conventional cylindrical implants were used. Half of the implants had a machined surface (Gcon) while in the other half, the surface was treated with particles of TiO2 followed by acid etching (Gexp). Surface characterization was assessed by scanning electron microscope (SEM), energy dispersive X-ray spectrometry (EDS), profilometry, and wettability. For the in vivo test, 12 implants of each group were implanted in the tibia of 6 rabbits, and were reverse torque tested after periods of 30 or 60 days after implantation. Following torque, SEM was utilized to assess residual bone-implant contact. The surface characterization by SEM showed a very homogeneous surface with uniform irregularities for Gexp and a small amount of residues of the blasting procedure, while Gcon presented a surface with minimal irregularities from the machining tools. Wettability test showed decreased contact angle for the Gcon relative to the Gexp. The Gexp removal torque at 30 and 60 days was 28.7%, and 33.2% higher relative to the Gcon, respectively. Blasting the surface with particles of TiO2 represents an adequate option for the surface treatment of dental implants, with minimal risk of contamination by the residual debris from the blasting procedure.

Key Words: dental implants, surface treatment, alumina, titanium micro particles, implant contamination

INTRODUCTION

Several physical and chemical features of titanium are relevant and suitable for biomedical applications. In particular, most of its intrinsic properties, such as biocompatibility, low specific weight, high strength-to-weight ratio, low modulus of elasticity, and excellent corrosion resistance, are favorable to the manufacturing of dental implants.1 Titanium surfaces can also be modified in an attempt to increase their biological properties. Such modifications are achieved by either 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 size, 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 industry, and their combination has shown improved biological activity of the titanium surface in terms of implant osseointegration relative to machined (as-turned) surfaces.3

The modification of the implant surface can thus bring benefits to the response of the surrounding bone tissue, accelerating the healing process and or improving the newly formed bone quality.3-5 Studies have shown that osseointegration is related to microgeometric features, such as the degree of surface roughness, and can also depend on factors such as physical and chemical surface properties.6,7 The latter may increase the surface wettability, enhance cell adhesion, and promote cell proliferation, increasing bone-to-implant contact formation.8 On the other side, macrogeometric features, such as the implant body shape and the design, height, density, and cutting ability of the threads, may affect the biomechanics of the implant-bone interlocking, possibly improving implant stability.8

Several types of chemical and physical surface treatments have been developed and marketed by dental implant manufacturers.9 However, there is still no consensus on what the optimal condition for peri-implant bone growth should be. It is known that the bone response can be influenced by the implant surface topography at the micrometer level, and some indication exists that a nanometric surface can also have an effect. However, the mechanisms behind an optimal bone response in relation to a given type of surface still remain largely unknown. Some biological 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 microroughness as well as by its physical-chemical surface properties.\textsuperscript{10–12}

Studies on the surface properties using photoelectric spectrometry, scanning electron microscopy, and other techniques have been described in literature.\textsuperscript{13} When changing the implant micromorphology, in vitro tests show that the surface energy is also modified, thereby potentially affecting in vivo cell migration, proliferation and cellular activity.

Surfaces known as SLA-types, which are produced by sandblasting with titanium particles followed by a strong acid-etching bath with a mixture of HCl/H\textsubscript{2}SO\textsubscript{4} at elevated temperature for several minutes, are widely utilized and have been well documented in the literature.\textsuperscript{14,15} These are moderately rough surfaces that usually present fine 2–4 μm micropits superimposed on the rough-blasted surface. Though well documented, the presence of residuals of alumina embedding on its surface due to the fabrication process has been regarded as a potential risk for long-term osseointegration.\textsuperscript{16–19} Alternatively, surfaces have been blasted with other biocompatible media such as calcium-phosphate bioactive ceramics\textsuperscript{20} and titanium oxide.\textsuperscript{21,22} The first comprises a resorbable medium that is actually bioactive, while the second method consists of particles that are made of the same biocompatible material as the implant. Though a wide literature body exists for the alumina-blasted/acid-etched surfaces relative to other surface modification techniques,\textsuperscript{14,15} a substantially smaller body of evidence exists for the resorbable blasting media and an even smaller one concerning the characterization and in vivo evaluation of TiO\textsubscript{2} blasted surfaces.

In unfavorable clinical situations, such as in the presence of poor quality bone, a fast and predictable osseointegration would be beneficial for allowing prosthetic rehabilitation. In the cases of insufficient bone quantity or anatomical limitations, or in the presence of local and systemic conditions representing a potential risk for long-term osseointegration implants with a rough surface show better bone apposition and BIC than implants with smooth surfaces.\textsuperscript{16–19,23,24}

The purposes of the present study were: (1) in vitro characterization of the physico-chemical features of the TiO\textsubscript{2} blasted surface by measuring the degree of roughness and testing the surface energy (wettability); and (2) in vivo study evaluating the surface behavior early after implantation, by measuring the torque removal and examining microscopically the residual bone formed on the surface of the implants.

**MATERIALS AND METHODS**

**In vitro study**

Thirty titanium disks of 5-mm diameter and 2-mm thickness were fabricated from the same bars used to manufacture the implants for in vivo testing. Fifteen disks had a machined surface and 15 disks had the surface treated following the same protocol as the conventionally marketed implant. These were prepared, packaged and sterilized with the same requirements and care as the implants. All materials were prepared by Implacil De Bortoli Company (São Paulo, Brazil).

**Protocol**

Five disks of each group were used for topography analysis and for quantification of percentage of surface covered by residual particles of the sandblasting by SEM and energy dispersive spectroscopy (EDS). Another 5 discs of each group were used for roughness measurements using a Mitutoyo Surftest 211 Profilometer (Mitutoyo Corporation, Tokyo, Japan); an average of 3 readings was performed for each surface. Five more disks of each group were used for the surface energy analysis, designed to check the flow rate of 5 μL of distilled water, applied with a micropipette on the sample. The wettability was estimated by measuring the total surface area wetted immediately after a droplet is placed on the surface. The surface tension was calculated by measuring the contact angle formed between the drop and the disk surface; it was scored as poor, good or complete wetting (Figure 1). For these evaluations, images were taken at 0, 15, 30, and 60 seconds with a high-resolution camera DSC-H9 (Sony, Tokyo, Japan). The images were analyzed using the program ImageTool version 5.02 for Microsoft Windows (The University of Texas Health, San Antonio, USA).

**In vivo study**

Twenty-four cylindrical self-tapping implants with internal hexagon packaged and ready for commercialization were used for in vivo testing. Twelve implants with a machined surface were used as the control group (Gcon) and 12 with the surface sandblasted with 140–180 μm titanium oxide (TiO\textsubscript{2}) particles at a 5 atm pressure for 1 minute, plus chemical treatment by maleic acid, were used as the experimental group (Gexp). Implant size was 4 mm in diameter and 8 mm in length.

**Protocol**

Six New Zealand white mature rabbits were used in this study. This study was approved by the ethics committee of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. The rabbits were anesthetized by intramuscular injection of ketamine (35 mg/kg; Agener Pharmaceutica, São Paulo, Brazil). Then, a muscle relaxant (Rompun, 5 mg/kg, Bayer, São Paulo, Brazil) and a tranquilizer (Acepran, 0.75 mg/kg, Univet, Santa Cruz Do Capibaribe, Brazil) were injected intramuscularly. Additionally 1 mL of local anesthetic (3% prilocaine-felypressin, Astra, Mexico City, Mexico) was injected subcutaneously at the site of surgery to improve analgesia and control bleeding. A skin incision with a periosteal flap was used to expose the bone.
in the proximal tibia. The preparation of the bone site was done with burs under copious saline irrigation. Two test and 2 control implants for each rabbit were inserted into the tibiae. It was decided to use the tibia as implant site for the simplicity of surgical access. The implant insertion was performed by hand. The insertion torque of the implants was controlled using a manual torque meter. The periosteum and fascia were sutured with catgut and the skin with silk. Postoperatively, a single dose of 600 000 IU benzetacil was used. After surgery the animals were placed in individual cages with 12-hour cycles of light, controlled temperature (21°C) and ad libitum diet normally used by the laboratory. No complications or deaths occurred in the postoperative period. Three animals were sacrificed after 4 weeks and the other three, at 8 weeks. All animals were euthanized with an intravenous overdose of ketamine (2 mL) and xylazine (1 mL). A total of 24 implants were retrieved. The biological specimen processing was made immediately after removal of the tibiae, these were taken to the Torque Testing Machine – CME (Técnica Industrial Oswaldo Filizola, Guarulhos, Brazil), which is fully controlled by the software, DynaView Torque Standard/Pro M (Hampshire, England). These implants were then fixed in formalin solution 10% for 72 hours, and then processed for histology. The specimens were dehydrated in an ascending series of alcohol rinses and placed in the desiccator, and then coated with a layer of gold. Micrographs were obtained using scanning electron microscope (SEM) model XL30 (Philips, Eindhoven, The Netherlands), by a series of images in the secondary electrons (SE) to forming an image of the surface. The SEM evaluation was conducted at various magnifications and the interfacial residual bone tissue on the implant surface assessed.

Data Analysis

For comparison between groups at each time in vivo, statistical analysis was performed by multiple paired t-tests considering the animal number per time in vivo as the statistical unit. For comparing each experimental group at different times in vivo, t-tests assuming equal variances were utilized. All evaluations were conducted at the 95% level of significance.

RESULTS

In Vitro Analysis

The analysis by SEM of the group Gexp, at different magnification, showed areas presenting large topographical uniformity, which is characteristic of surfaces that are first blasted and then acid treated. A few residues of particles (arrows) used for the sandblasting procedure are present (Figure 2b). Since these samples were previously blasted with particles of titanium oxide, in-depth texturing (ripples) was observed. SEM evaluation of Gcon showed a smoother pattern compared to the blasted surface that is commonly observed in as-machined surfaces (Figure 2a). EDS evaluation of both groups showed large concentrations of Ti and no presence of contaminants, proving that particles present in the surface of group Gexp were of the sandblasting procedure. The percentage of implant surface covered by particles was 5.8 ± 2.4%. The average roughness was 0.159 µm for Gcon and 0.699 µm for Gexp. The surface wettability results are presented in Figure 3, where the Gcon depicts a steeper decline in value over time relative to the Gexp, which presented a more subtle decrease. Wettability can also be observed in the image of the liquid on the samples 60 seconds after dropping (Figure 3b).

In Vivo Analysis

The surgical procedures were uneventful. The mean insertion torque of the implants was 18 ± 3 Ncm. All animals presented appropriate healing during the first week following the surgical procedure. Postsurgical inspections for 2 weeks postoperatively indicated the absence of infection or inflammation. The biomechanical testing indicated that all implants integrated based on the values obtained for each implant’s removal torque. The mean values ± standard deviations are presented in Figure 4. The paired statistical observation showed that the Gexp presented significantly higher torque values than the Gcon at 30 and 60 days in vivo (P < .01 and P < .03, respectively). When each group was compared as a function of time in vivo, no significant differences were detected for both
Gcon ($P > .35$) and Gexp ($P > .08$) despite the overall increase in torque values as time elapsed in vivo.

Histological analysis showed an incomplete cell organization at 30 days in the group Gcon, and a better organization, with greater quantity of cells in the group Gexp (Figure 5a,b). At 60 days, the group Gcon showed a beginning of cellular organization, but with reduced quality and quantity as compared to the group Gexp, that showed mature bone with a lamellar organization (Figure 6a,b).

The implant surface evaluation by SEM after removal torque test at 30 days, showed a minimal presence of bone at the Gcon surface, whereas a larger amount was observed at the Gexp group mainly in the inner diameter of the threads (Figure 7). At 60 days, the difference in the amount of bone at the Gcon and Gexp groups was more remarkable than at 30 days, as well as differences in bone morphology were evident. For the Gcon, the remaining bone at the interface was irregular and not organized as compared to the Gexp (Figure 8).

**DISCUSSION**

Over the past decades, a multitude of in vivo studies examined the effect of the implant surface on the bone healing and apposition. Modifications in implant surface morphology and roughness have been initially attempted aiming not only to hasten the host-to-implant response but also to increase the level of mechanical interlocking between bone and implant surface, thus improving the initial stability, and subsequent stress dissipation during functional loading. Histology-based investigations have shown that surface texturing created by blasting led to greater bone-implant contact as compared with the machined surface, which is a desirable response for improving the overall system biomechanics. Blasting the implant surface with gritting agents made of materials other than the implant core material may change the surface composition and the implant biocompatibility. Abrasive blasting increases the surface roughness, as well as the metal surface reactivity. With the use of a blasting material like Al2O3, a potential risk of contamination by remnants of blasting particles with dissolution of aluminium ions into the host tissue cannot be excluded. It has been reported that Al ions may inhibit normal differentiation of bone marrow stromal cells and normal bone deposition and mineralization, and aluminium has been shown to induce net calcium efflux from cultured bone. Moreover, aluminium may compete with calcium during the healing of implant bed. Aluminium has also been shown to accumulate at the mineralization front and in the osteoid matrix itself. Therefore other alternative methods were developed to sandblasting in order to roughening the implant surface, such as the use of resorbable particles based on calcium and particles of TiO2 both of which are unproblematic when small residues remain deposited after surface treatment procedures.

The effects of sandblasting the implant surface with titanium oxide as an alternative to aluminium oxide has been investigated previously. The research protocols took into account biomechanical (removal torque), interfacial, and histological analyses as well as histomorphometric and microhardness measurements. Only 1 study observed and...
analyzed the specimens using both scanning electron microscopy and histomorphometry, as well as removal torque test in dogs. This study reported that implants blasted with titanium-dioxide-particles had a better anchorage than implants with a machine-produced surface, in spite of no difference in bone-implant contact.

In the present study, SEM analysis showed that the surface treatment by sandblasting leaves residues deposited mainly on the bottom of the undulations created, that even with the subsequent procedures, such as acid conditioning, cleaning and passivation of implants are not fully removed. The EDS analysis proved that these residues were made of titanium, a material that has demonstrated great osteoconductive potential, assisting in cell stimulation, and improving the quality and quantity of ossification around the implants. Bone conduction is often seen with materials of high biocompatibility such as commercially pure (commercially pure) titanium.

Different studies have reported that surface acid etching reduces the concentrations of C, Ti, and N, but increases the amount of oxygen, revealing a more oxidized surface compared to baseline substrate alloy characteristics. Thus, either grit-blasting alone or in combination with a subsequent acid-etching protocol not only alters surface texture but also surface chemistry and wettability, presenting the potential to alter the early interaction between the host biological fluids and implant surface. The application of maleic acid after sandblasting the surface promotes the roundness of the undulations created, making the surface topography more uniform.

The surface characterization results are in agreement with previously published work, where a smoother surface was observed for the machined surfaces and a moderately rough surface was evident for the TiO2 blasted surface. From a surface chemistry perspective, no chemical elements other than the expected in the titanium alloy utilized for the implant fabrication were detected for both groups. However, when surface wettability was evaluated, higher wetting was observed for the machined compared to textured surface. Studies
related that feature known to be of utmost importance during the initial stages of osseointegration as textured surfaces’ ability to attract and retain the blood clot responsible for the subsequent osteogenic cascade is increased by higher surface wetting characteristics.\textsuperscript{38,52} A drop on a rough and hydrophobic surface can adopt 2 configurations: a Wenzel (complete wetting) and a Cassie–Baxter configuration (partial wetting),\textsuperscript{53} as presented in Figure 9a and b, respectively. In both cases, even if locally, the contact angle does not change (angle of Young), an increase in the apparent contact angle of the drop is observed. For a superhydrophobic surface, the fundamental difference between the 2 models is the hysteresis value. For a low roughness, a strong hysteresis being able to reach 100° (Wenzel) is observed and attributed to an increase in the substrate surface in contact with the drop. It’s possible that this characteristic stabilize faster and more strongly the clot at

\textbf{FIGURES 7 AND 8.} FIGURE 7. Histological pictures showing bone maturation and the bone-to-implant contact (BIC) after 30 days. Sequence (a) group Gcon and (b) group Gexp. Magnification ×32, ×500, and ×1000, respectively. FIGURE 8. Images of the BIC in the Gcon and Gexp after 60 days. Sequence (a) group Gcon and (b) group Gexp. Magnification ×32, ×500, and ×1000, respectively.
the nature of the implant tissue interface. In this study, both surface biocompatibility and osseoconductive properties were confirmed by the biomechanical tests and the subsequent scanning electron micrographs that depicted intimate interaction between newly formed bone and either implant surfaces. Such interaction was more pronounced for the textured surface as compared to machined, indicating that the mechanical tests results may have been a synergy of the mechanical interlocking between bone and implant surface and the higher bone formation compared to the machined surface. The reverse torque values may appear rather high even for the machined surface. This might depend on the experimental model chosen. In fact, the cortical bone of the rabbit tibia is very compact and may achieve a good mechanical interlocking between bone and implant surface and subsequently transfer the degree of the force of osseointegration and the characteristics of the bone around of the surface after 30 and 60 days. In fact, previous research had shown that the surface characteristics were important in influencing the bone–implant contact percentages and statistically significant differences were observed in different implant surfaces. Histomorphometric and removal torque measurements are 2 representative tests in studying the nature of the implant tissue interface. In this study, both surface biocompatibility and osseoconductive properties were confirmed by the biomechanical tests and the subsequent scanning electron micrographs that depicted intimate interaction between newly formed bone and either implant surfaces. Such interaction was more pronounced for the textured surface as compared to machined, indicating that the mechanical tests results may have been a synergy of the mechanical interlocking between bone and implant surface and the higher bone formation compared to the machined surface. The reverse torque values may appear rather high even for the machined surface. This might depend on the experimental model chosen. In fact, the cortical bone of the rabbit tibia is very compact and may achieve a good mechanical interlocking with the implants. However, the aim of the present study was not to estimate parameters values that could be directly transferred to the patients, but to compare 2 different treatments of titanium dental implants for rapid osseointegration. Within the limitations of this study, the titanium oxide blasting displayed a positive effect on osseointegration and on the biomechanical features of the implants. The histological results confirmed the hypothesis that the presence of residual blasting titanium particles on the surface of dental implants does not affect the osseointegration of titanium dental implants. The results demonstrated that even with a low wettability (high contact angle) the osseointegration was very effective, though further studies are needed to get more insight into this topic.

**Conclusion**

Within the limitations of this study, the titanium oxide blasting displayed a positive effect on osseointegration and on the biomechanical features of the implants. The histological results confirmed the hypothesis that the presence of residual blasting titanium particles on the surface of dental implants does not affect the osseointegration of titanium dental implants. The results demonstrated that even with a low wettability (high contact angle) the osseointegration was very effective, though further studies are needed to get more insight into this topic.

**ABBREVIATIONS**

EDS: energy dispersive X-ray spectrometry
Gexp: surface treated with particles of TiO2 followed by acid etching
SEM: scanning electron microscope

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