

Histological and biomechanical effects of implant surfaces sandblasted with titanium dioxide microparticles: An experimental study using the rabbit tibia model

Abstract

Objective

The objective of this study was to investigate the effect of sandblasted, large-grit, acid-etched (SLA) implant surfaces treated with titanium dioxide (TiO₂) microparticles on the implants' stability and resistance to reverse torque.

Materials and methods

Six rabbits received 24 cylindrical dental implants and were placed in two groups ($n = 3$ per group): control group, with smooth surfaces; and test group, with the SLA surface treated with TiO₂ microparticles. All of the animals were sacrificed after four weeks. Half of the implants (one per animal from each group) were used to test removal torque values and half of them were used for the histological analysis.

Results

Reverse torque was significantly different between the groups ($p = 0.0001$). The histological analysis showed higher degrees of bone organization in surface samples from the test group.

Conclusion

Results indicate that blasting implant surfaces with TiO₂ particles is an appropriate treatment option, with minimal risk of contamination by residual debris from the procedure.

Keywords

Implant surface, osseointegration, removal torque, titanium dioxide microparticles.

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Introduction

Per-Ingvar Brånemark, a Swedish professor, demonstrated that osseointegration of titanium implants is such that the bone remains in close contact with the implant surface without any intervention by the connective tissue, although the titanium dioxide (TiO₂) layer interacts directly with the bone tissue.¹ The physical and chemical features of titanium, particularly its intrinsic properties, such as biocompatibility, low specific weight, high strength–weight ratio, low modulus of elasticity, and excellent corrosion resistance, are favorable for the manufacture of dental implants.² Furthermore, titanium surfaces can be modified in an attempt to enhance their biological properties.³ Such modifications are achieved by adding a coat 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 applying chemical treatments and/or physical ones, such as laser.⁴ Among these, blasting and acid etching have been the most widely used. In addition, their combination has shown improved biological activity of titanium surfaces in terms of implant osseointegration relative to smooth (machined) surfaces.⁵

The modification of the implant surface can thus have benefits regarding the response of the surrounding bone tissue, accelerating the healing process and/or improving the quality of the newly formed bone.^{5–7} Studies have shown that osseointegration is related to microgeometric features, such as the degree of surface roughness, and to factors such as the physical and chemical properties of surfaces.^{7,8} Rough surfaces were found to stimulate osteoblastic gene expression and to enhance bone formation and bone implant fixation.^{9,10} While an associated inflammatory response was reported,¹¹ the overall success rate was satisfactory, with the majority of implants yielding good osseointegration and stability one year after surgery.¹²

Dental implant manufacturers have developed and marketed implants with several types of chemical and physical surface treatments.¹³ However, there is still no consensus on what the optimal conditions for periimplant bone growth are. It is known that bone response can be influenced by implant surface topography at the micrometer level, and it has been hypothesized that a nanometric surface can also have an effect.¹⁴ Notwithstanding, the mechanisms be-

hind an optimal bone response to a given type of surface still remain largely unknown.

Surfaces known as SLA (sandblasted, large-grit, acid-etched) are produced by blasting with microparticles of some materials followed by acid etching. Alumina is one of the most widely used materials, but some authors have highlighted some features of alumina blasting that could compromise osseointegration (e.g., particle detachment during the healing process and absorption by the surrounding tissues).¹⁵ The presence of alumina residues on implant surfaces due to the manufacturing process has been regarded as a potential risk, compromising long-term osseointegration.^{16,17} Alternatively, TiO₂ is used as a blasting material and has shown interesting results in experimental studies. Particularly, TiO₂-blasted implants were associated in humans with a significant enhancement of bone-to-implant contact (BIC) when compared with machined surfaces.^{18–20} Under unfavorable clinical conditions, such as in the presence of poor-quality bone, fast and predictable osseointegration would be beneficial, allowing prosthetic rehabilitation. In the case of insufficient bone quantity or anatomical limitations, or in the presence of local and systemic conditions that could compromise long-term osseointegration, implants with a rough surface show better bone apposition and BIC than do those with smooth surfaces.^{21,22} Therefore, the aim of the present *in vivo* study was to evaluate the behavior of surfaces shortly after implantation by measuring removal torque and analyzing histological parameters.

Materials and methods

Twenty-four cylindrical self-tapping implants with internal hexagon packaged and ready for sale were used for *in vivo* testing. Twelve implants with a machined surface (**Fig. 1**) were used in the control group (C group). Twelve implants with surfaces sandblasted with 50–150 µm TiO₂ microparticles at a 5 atm pressure for 1 min, ultrasonically cleaned with an alkaline solution, rinsed in distilled water and then conditioned with maleic acid (**Fig. 2**) were used in the test group (T group). The implants (Implacil De Bortoli, São Paulo, Brazil) were 4 mm in diameter and 8 mm in length.

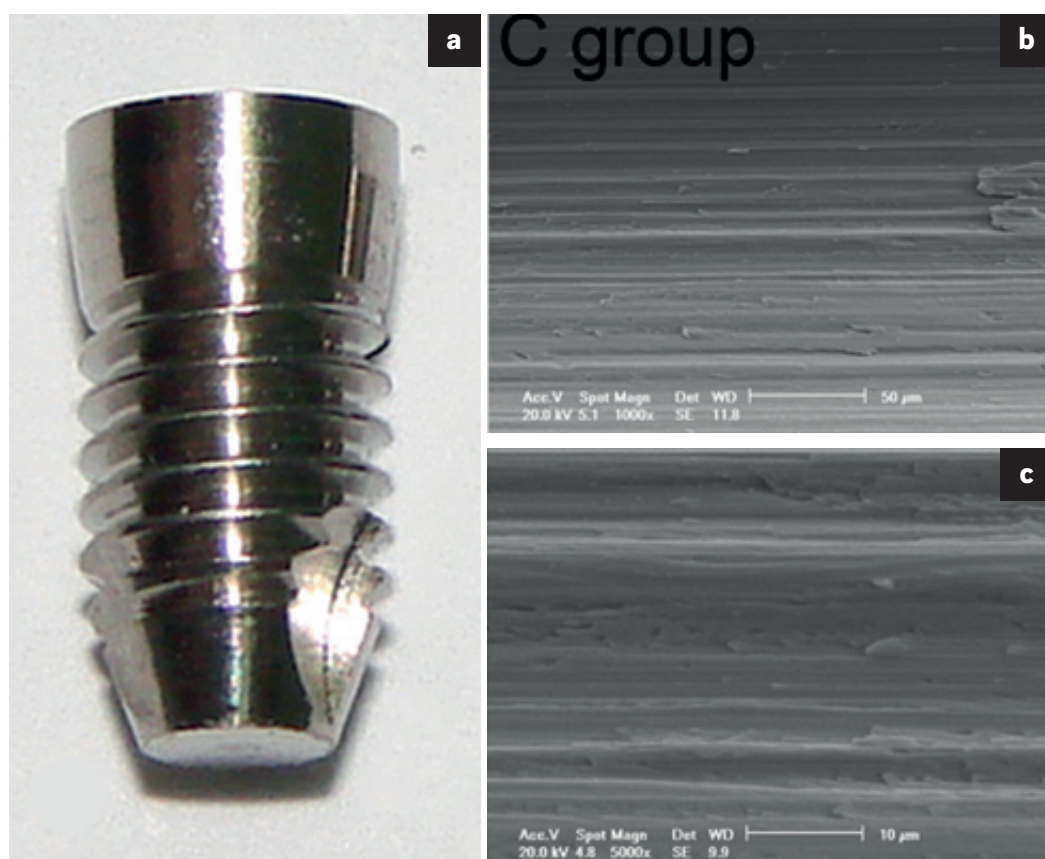
Six mature New Zealand white rabbits were used in this study. This study was approved by the Ethics Committee (#004-09-2015) of the

Figs. 1a–c

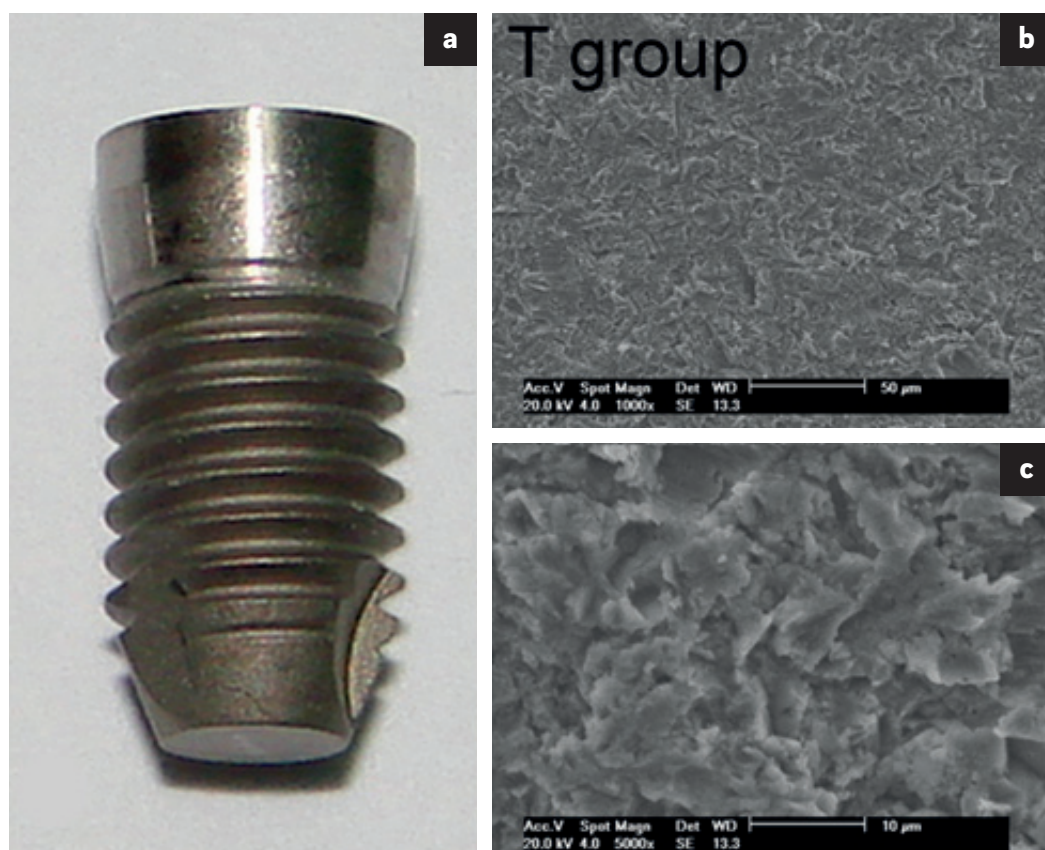
(c) Image of the implant used as control (C group), with smooth surface.
(b & c) SEM images of the surface at 1,000× and 5,000× magnification.

Figs. 2a–c

(c) Image of the implant used as test (T group), with SLA surface.
(b & c) SEM images of the surface at 1,000× and 5,000× magnification.



Figs. 1a–c



Figs. 2a–c

Figs. 3 & 4

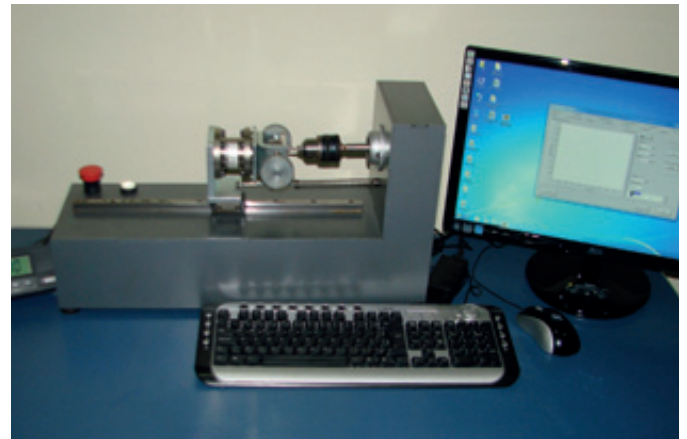


Fig. 3
Image of the implants inserted into the tibia.

Fig. 4
Image of the computerized torque machine used in the removal torque test.

Itapiranga Faculty of Veterinary Medicine, Itapiranga, Brazil. The rabbits were anesthetized by intramuscular ketamine (35 mg/kg; Agener Pharmaceutica, Brazil). Thereafter, a muscle relaxant (Rompum 5 mg/kg, Bayer, Brazil) and a tranquilizer (Acepran 0.75 mg/kg, Univet, Brazil) were injected intramuscularly. Additionally, 1 mL of local anesthetic (3% prilocaine-felypressin, Astra, 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 implants were inserted into the tibial metaphysis of each rabbit (**Fig. 3**), one most proximal at 5 mm from the articulation and the other 10 mm to the distal, thus avoiding differences in bone typology in this area. The implant position was randomized for each animal at www.randomization.com. The tibia was chosen as the implant site because it provides easier surgical access. The implant insertion was performed by hand with a torque of < 20 N until locking of the implant in the opposite cortical portion of the osteotomy, as part of the implant shoulder just out in relation to the top of the cortical bone crest, thereby avoiding excessive compression of the bone due to implant design. The periosteum and fascia were sutured with catgut and the skin with silk. Postoperatively, a single dose of 600,000 IU of benzathine penicillin (Benzetacil, Eurofarma Laboratórios, Rio de Janeiro, Brazil) was used. After surgery, the animals were placed in individual cages with 12-h cycles of light, controlled temperature (21 °C), and food and water *ad libitum*. No complications or deaths occurred in the postoperative period. All of the animals were euthanized after four weeks using an intravenous overdose

of ketamine (2 mL) and xylazine (1 mL). A total of 24 implants were retrieved. The implants of all right tibiae were immediately analyzed using a torque-testing machine (CME, Técnica Industrial Oswaldo Filizola, Guarulhos, Brazil), which was fully controlled by DynaView Torque Standard/Pro M software (**Fig. 4**).

All of the implants of the left tibiae were used for histological analysis and were placed in 10% formalin after removal and taken to the Biotecnos Laboratory (Santa Maria, Brazil). After the fixation period, they were dehydrated in an ascending series of alcohols and embedded in glycol methacrylate resin (Technovit 9100 VLC, Kulzer, Hanau, Germany) to produce undecalcified sections. Undecalcified cut and ground sections that contained the central part of each implant and had a final thickness of 15 µm were produced using a macro-cutting and -grinding system (Iso-met 2000, Buehler, Braunschweig, Germany). The sections were stained with picro-sirius-hematoxylin, and histomorphometric analysis was then carried out. The specimens prepared for the analysis of the tissue around the implant were examined under a light microscope (EOS 200, Nikon, Tokyo, Japan). After digitizing the phase of each specimen under a light microscope, the percentage of bone-to-implant contact (BIC%) was measured using the Image Tool software for Microsoft Windows (Version 5.02). BIC% was calculated as the percentage of the total length of bone in direct contact with the implant surface, from the first crestal bone contact to the most apical contact.

The statistical analysis was performed using the *t*-test for comparison between groups. Two correlation measurements were used to assess the relationship between the groups: Pearson's correlation coefficient (with $-1 < R < 1$; when *R* is

Fig. 5
Removal torque values (N cm)
at four weeks in both groups.

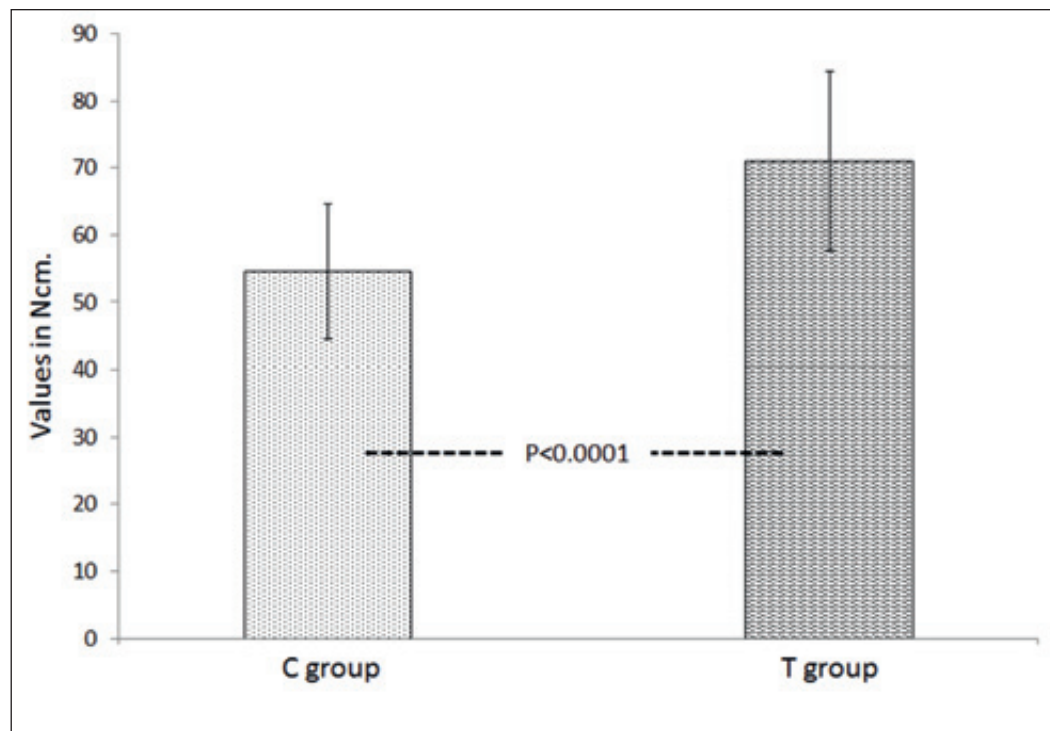


Fig. 5

close to ± 1 this indicates that the variables are correlated; however, the relationship is linear) and Spearman's rank correlation coefficient, similar to Pearson's correlation coefficient, with $-1 < R < 1$. This measurement was more comprehensive because we assessed whether the relationship between the variables was nonlinear. All of the tests were performed using specific software (MedCalc, MedCalc Software, Belgium). The level of significance was set at $\alpha = 0.05$.

Results

The surgical procedures were uneventful and all of the animals presented appropriate healing within the first weeks after surgery. Inspections made during two postoperative weeks indicated no infection or inflammation. The biomechanical tests indicated osseointegration of all of the implants, but torque after four weeks was higher in the T group (71.0 ± 13.4 N cm; median of 73.5) than in the C group (54.5 ± 10.0 N cm; median of 56.5). The mean \pm standard deviations and the statistical comparison are presented in **Figure 5**. The paired statistical tests showed that torque was significantly higher in the T group than in the C group at four weeks ($p < 0.0001$).

BIC% was higher in the T group ($64.8 \pm 7.4\%$; median of 66.0) after four weeks than in the C group ($50.4 \pm 7.9\%$; median of 49.5). These

data and statistical significance ($p = 0.0005$) are shown in **Figure 6**. The new bone formed around the implants in the C group was not completely mineralized (**Fig. 7**). In the T group, however, better organization and mineralization were found after four weeks (**Fig. 8**) and there was better stimulation of the medullary bone portion (**Fig. 9**).

The Kolmogorov–Smirnov test identified that only the BIC% of the T group had nonparametric data. Thus, the correlation between reverse torque and BIC% (machined) was determined by Pearson's correlation coefficient ($R = -0.52$; $p = 0.08$; 95% CI $[-0.84-0.07]$), whereas the correlation between reverse torque and BIC% (treated) was determined by Spearman's correlation coefficient ($R = 0.08$; $p = 0.79$; 95% CI $[-0.51-0.62]$). The statistical data are summarized in **Table 1**.

Discussion

Over the past decades, several *in vivo* studies have examined the effect of implant surfaces on bone healing and apposition.^{23, 24} Modifications in implant surface morphology and roughness were initially attempted to hasten host response to implants and to increase the level of mechanical interlock between the bone and implant surface, thus improving initial stability and sub-

Fig. 6

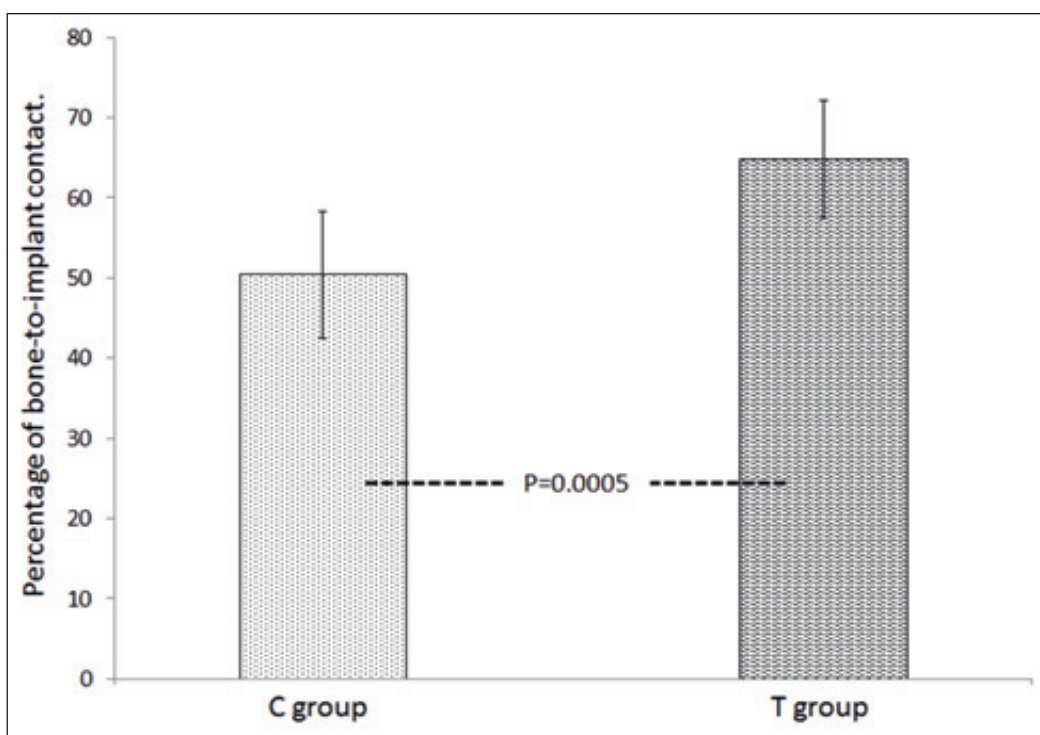


Fig. 6

BIC values (%) at four weeks in both groups.

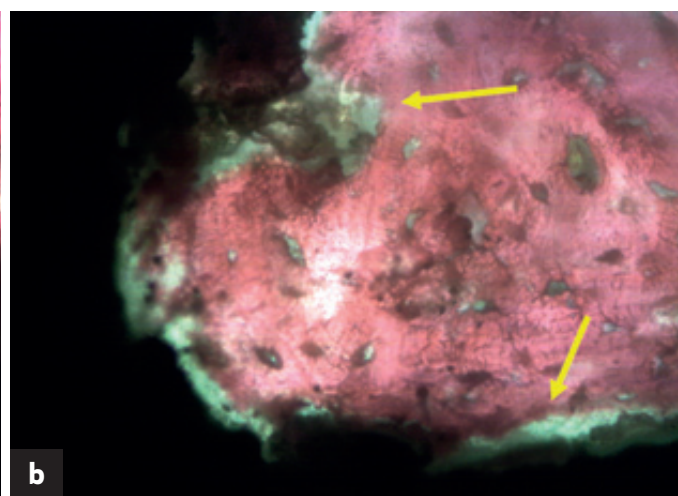
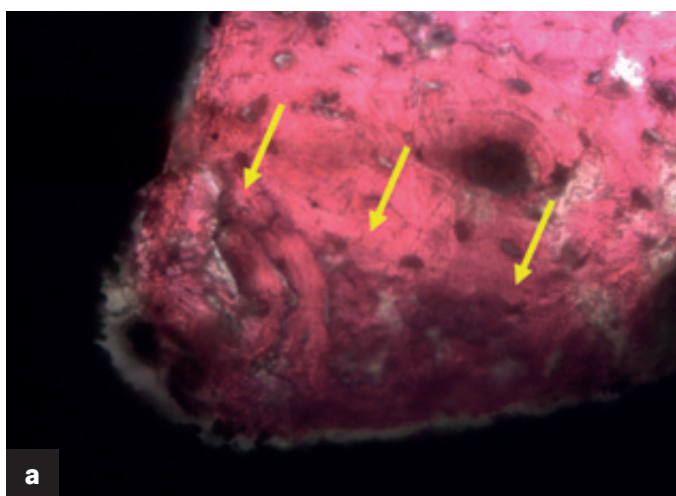
Figs. 7a & b

Histological images showing bone maturation and mineralization in the C group after four weeks, with new bone formation around the implants showing incomplete mineralization. (a) 200× and (b) 400× magnification. Staining with picro-sirius-hematoxylin.

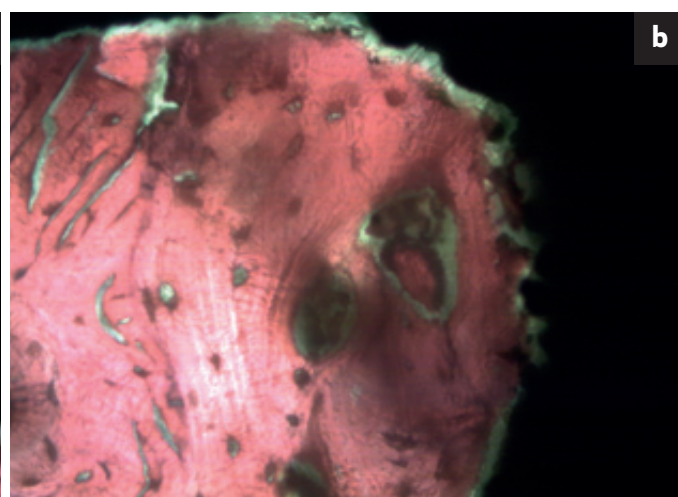
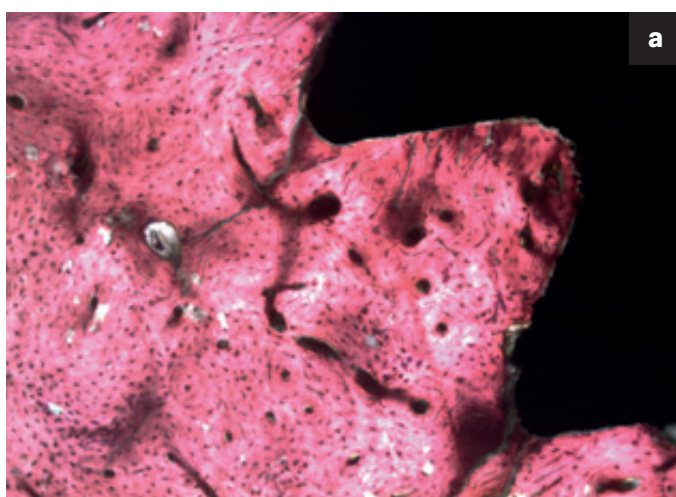
Figs. 8a & b

Histological images showing bone maturation and mineralization in the T group after four weeks, with more advanced new bone formation around the implants in the new bone organization areas. (a) 100× and (b) 400× magnification. Staining with picro-sirius-hematoxylin.

Figs. 7a & b



Figs. 8a & b



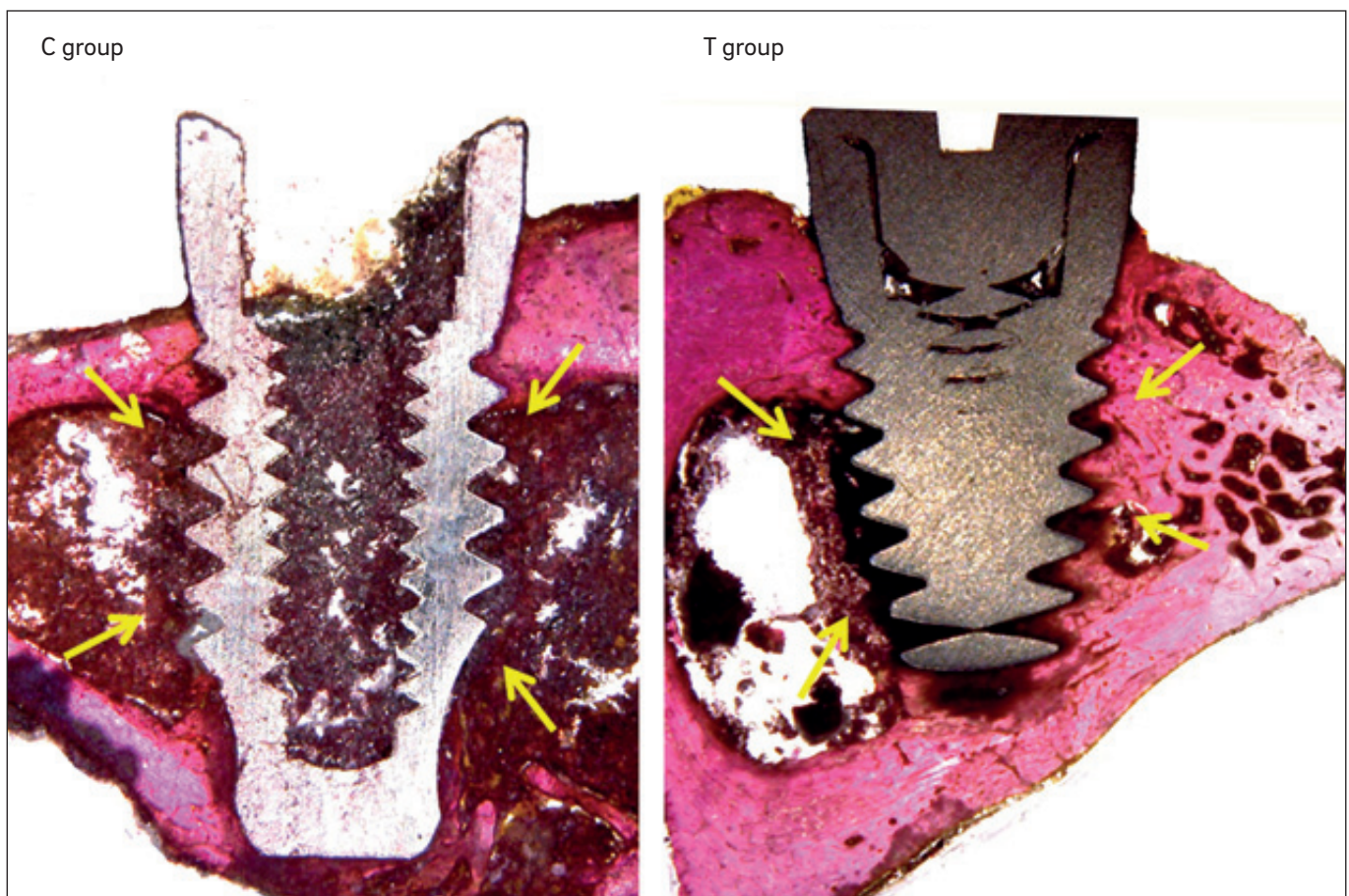


Fig. 9

Fig. 9

Histological images showing bone maturation and the BIC after four weeks. There was visibly better stimulation of the medullary bone portion in the T group in comparison to the C group (yellow arrows).

Table 1

Mean \pm SD and median (reverse torque and BIC%) values at baseline and at eight weeks.

| Groups | Reverse torque (N cm) | | BIC (%) | | Coefficient of correlation |
|---------|-----------------------|------------|----------------|---------|----------------------------|
| | Mean \pm SD | Median | Mean \pm SD | Median | |
| Control | 54.5 \pm 10.0 | 56.5 | 50.4 \pm 7.9 | 49.5 | 0.08 |
| Test | 71.0 \pm 13.4 | 73.5 | 64.8 \pm 7.4 | 66.0 | 0.79 |
| P-value | | < 0.00001* | | 0.0005* | |

Table 1

* Between-group comparisons (Wilcoxon's test; significance level: $p < 0.05$).

sequent stress dissipation during functional loading.^{25, 26}

Histological investigations have shown that the surface texture created by blasting leads to greater BIC than that of machined surfaces,²⁷ which is a desirable response, as it allows improvement of the overall biomechanics of the system. Blasting the implant surface with gritting agents made of materials other than alumina may change the surface composition and implant biocompatibility.²⁸ Abrasive blasting increases surface roughness and metal surface reactivity.²⁸

With the use of a blasting material such as alumina, a potential risk of contamination by remnants of blasting particles, with dissolution of aluminum ions into the host tissue, cannot be excluded.²⁸ It has been reported that aluminum ions may inhibit normal differentiation of bone marrow stromal cells and normal bone deposition and mineralization,^{29–31} and aluminum has been shown to induce net calcium efflux from the cultured bone.³² Moreover, aluminum may compete with calcium during the healing of the implant bed. Aluminum has been shown to ac-

cumulate at the mineralization front and in the osteoid matrix itself.³³ Therefore, other alternative sandblasting methods were developed in order to roughen the implant surface, such as the use of resorbable particles based on calcium³⁴ and TiO₂,^{35, 36} both of which are unproblematic if small residues remain after surface treatment procedures.

The effects of sandblasting the implant surface with titanium oxide as an alternative to aluminum oxide have been investigated previously.^{19, 20, 27, 28, 37–40} The research protocols took into account biomechanical (removal torque), interfacial and histological analyses, as well as histomorphometric and microhardness measurements. Only one study observed and analyzed specimens using both scanning electron microscopy (SEM) and histomorphometry, as well as the removal torque test, in dogs.³⁷ This study demonstrated that implants blasted with TiO₂ particles had a better anchorage than implants with a machine-produced surface, in spite of there being no difference in BIC.³⁷

Animal models are essential in providing phenomenological information on biological reaction to endosseous implants.⁴¹ The removal torque test is among the *in vivo* mechanical tests commonly used to evaluate the strength of the interaction between the bone and implant surface.^{42–44} High resistance to implant removal encountered during these tests indicates good integration between the bone and implant surface, or in the case of porous materials, a high degree of bone ingrowth into the pores of the implant.⁴⁴ The present study evaluated the extent of osseointegration and the characteristics of the bone around the surface within four weeks after implantation.

Previous research has shown that surface characteristics influenced BIC, with statistically significant differences on different implant surfaces.⁴¹ Histomorphometric and removal torque

measurements are two representative tests used to assess the nature of the implant–tissue interface.⁴⁵ In this study, both surface biocompatibility and osteoconductive properties were confirmed by the biomechanical tests. Such interaction was more pronounced for the textured surface compared with the machined one, indicating a possible synergistic interaction of the mechanical interlock between the bone and implant surface and higher bone formation compared with the machined surface. The reverse torque values may appear rather high even for implants with a machined surface. This has to do with the experimental model chosen. In fact, the cortical bone of rabbit tibia is very compact and may firmly interlock with the implants. However, the aim of the present study was not to estimate parameter values that could be directly transferred to patients, but to compare two different surfaces using both *in vitro* and *in vivo* approaches. The results confirm that TiO₂-blasted surfaces allow for greater osteoconductivity and accelerated bone formation compared with machined surfaces and are therefore recommended for anticipated loading protocols.

Conclusion

Despite the limitations of this study, TiO₂ blasting displayed a positive effect on osseointegration and on the biomechanical features of the implants. The histological results confirmed the hypothesis that the SLA surface using blasting with TiO₂ microparticles positively affects the osseointegration of titanium dental implants.

Competing interests

The authors declare that they have no conflict of interests related to this study.

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