A comparative evaluation between aluminium and titanium dioxide microparticles for blasting the surface titanium dental implants: an experimental study in rabbits

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Abstract

Objective: The aim of this study was to compare, through biomechanical and histological analysis, the aluminium (AlO₂) and titanium dioxide (TiO₂) microparticles for blasting during the sandblasting-acid surface treatment in titanium dental implants using a rabbit tibia model.

Materials and methods: Forty-eight commercially available titanium dental implants were divided into two test groups (n = 24 per group): implants with surface treated by AlO₂ followed by acid etching as control group (Con group) and implants with surface treated by TiO₂ followed by acid etching as test group (Test group). The implants were randomly installed in both tibias of eight rabbits and block samples were removed 4 and 8 weeks after implantation. Resonance Frequency Analyses were performed immediately after the implantation and at 8 weeks. Twelve implants of each group were removed to measure the reverse torque. The remaining implants were used for histological analysis. The data were compared using statistical tests (α = 0.05).

Results: In comparing the implant stability quotient at the two time points, no significant statistical differences were found (P > 0.05), as well as in the removal torque test at 8 weeks after implant placement, no found significant difference between the two groups was tested. Histomorphometric analysis showed a high degree of bone organization in all samples with no significant difference between groups in the bone-to-implant contact (P > 0.05).

Conclusion: Within the limitations of this study, the results indicate that the media of surface blasting (AlO₂ or TiO₂ microparticles) did not show significant differences in the tested parameters for assessing the osseointegration of the implants.

Success rates of titanium dental implants-based therapy in dentistry have been documented to be over 98% [Buser et al. 1997; Mangano et al. 2010]. Implant success is strictly related to the osseointegration process that has been defined as the formation of a direct bone-implant interface with no intervening soft tissues [Branemark et al. 1969]. Titanium surfaces can also be modified to increase their biological properties. Such modifications are achieved by either adding a coating consisting of different types of bioactive substances or 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 [Wennerberg & Albrektsson 2009]. 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 as compared to machined (turned) surfaces [Novaes et al. 2010].

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 [Novaes et al. 2010; Wennerberg & Albrektsson 2010]. 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 [Sul et al. 2005; Le Guéhéneuc et al. 2007]. The macrogeometric design such as the implant body shape, height, density...
and cutting ability of the threads may affect the biomechanics of the bone–implant interlocking, possibly improving implant stability (Hallgren et al. 2003).

Several types of chemical and physical surface treatments have been developed and marketed by dental implant manufacturers (Binon 2000). 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 indications exist that a nanometric surface can also have an effect (Pan et al. 2012). 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 (Schliephake et al. 2006, 2009a,b, Lutz et al. 2010).

Surfaces known as sandblasting acid (SLA) types, which are produced by sandblasting with titanium particles followed by a strong acid etching bath with a mixture of HCl/H2SO4 at elevated temperature for several minutes, are widely utilized and have been well documented in the literature (Li et al. 2002; Esposito et al. 2005; Kim et al. 2015). These are moderately rough surfaces that usually present fine 2–4-μm micropits superimposed on the rough-blasted surface. Although 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 (Piattelli et al. 2003; Gehrke et al. 2014, 2015). Alternatively, surfaces have been blasted with other biocompatible media such as calcium phosphate bioactive ceramics (Piattelli et al. 2002; Schliephake et al. 2006, 2009a) and titanium oxide (Zinger et al. 2004; Gehrke et al. 2014, 2015). 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. Even though a wide literature body exists for the alumina-blasted/acid-etched surfaces relative to other surface modification techniques (Li et al. 2002; Esposito et al. 2005), 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 TiO2-blasted surfaces.

The purpose of this study was to compare, through biomechanical and histological analyses, the effects of aluminium (Al2O3) and titanium dioxide (TiO2) microparticles for blasting used to produce the SLA surface treatment of two commercially available titanium dental implants, using a rabbit tibia model.

Material and methods

Forty-eight cylindrical dental implants were used for this study (Fig. 1). They were divided into two groups of 24 implants each: a control group of implants (Con group) with SLA surface that is produced using the Al2O3 microparticles for blasting and subsequent acids conditioning (Straumann, Basel, Switzerland) and a test group of implants (Test group) produced using TiO2 microparticles for blasting and subsequent acid conditioning (Implacol De Botoli, São Paulo, Brazil). Implant size was 4 mm in diameter and 8 mm in length. All implants used in this study were purchased from the respective distributors of each product.

Animals and surgical procedure

Eight New Zealand white adult rabbits weighing approximately 4 kg were used in this study. The experiment protocol was designed in accordance with the Spanish and European guidelines for animal experiments. The experiment was approved by the Ethics Committee for Animal Research of the University of Murcia (Spain), in accordance with the European Union Council Directive of Feb. 1, 2013 (R.D.53/2013). The rabbits were anaesthetized with an intramuscular injection of tiletamine/zolazepam 15 mg/kg (Zoletil 50; Virbac, Madrid, Spain) and xylazine 5 mg/kg (Rompun; Bayer, Leverkusen, Germany). Before surgery, the shaved skin over the area of the proximal tibia was washed with Betadine®; Meda Manufacturing, Burdeos, France. Ketamine hydrochloride (Ketolar®, Pfizer, Madrid, Spain) was administered as an anaesthetic at 50 mg/kg IM. A pre-operative antibiotic (Amoxicillin; Pfizer, Barcelona, Spain) was administered intramuscularly. Additionally, 1 ml of local anaesthetic (3% Prilocaine-felypressin, Astra, Mexico) was injected subcutaneously at the site of surgery to improve analgesia and to control bleeding. A skin incision with a periosteal flap was used to expose the bone of both proximal tibias. The bone site was prepared with burs under copious saline irrigation. Three implants were inserted in each tibia using a computer-generated random sequence (www.randomization.com). The implants were positioned at the same level as the marginal border, that is, at bone level, and were fixed bicortically. The insertion torque of the implants was controlled using a manual torque metre and did not exceed 20 ± 3 Ncm; the implant stability quotient (ISQ) was then measured as described later. The periosteum and fascia were sutured with 5-0 vicryl sutures and the skin with silk sutures. Postoperatively, a single dose of 600,000 IU Benzacetil was used. After surgery, the animals were placed in individual cages with 12-h cycles of light/dark, controlled temperature (21°C) and the ad libitum diet that is normally used by the laboratory. No complications or adverse events occurred during the postoperative period. All animals were euthanized with an intravenous overdose of ketamine (2 ml) and xylazine (1 ml); four animals were killed at each time point: 4 weeks and 6 weeks after the implantations. Both tibias were removed, placed in 10% formalin solution and immediately taken to the laboratory (Biotecnos, Santa Maria, Brazil) for analysis.

Resonance Frequency Analysis

Resonance Frequency Analysis (RFA) was used to measure the implant stability in all rabbits. A Smartpeg™ (Integration Diagnostics AB, Göteborg, Sweden) was screwed into each implant and tightened to approximately 5 N. The ISQ values were measured by Osstell™ Mentor [Integration Diagnostics AB]. The transducer probe was aimed at the small magnet at the top of the Smartpeg at a distance of 2 or 3 mm and held stable during the pulsing until the instrument beeped and displayed the ISQ value. For RFA, the implants were measured immediately after
the installation and 8 weeks after the implant installation. The ISQ values were measured in two perpendicular directions (proximal to distal and lateral to medial), and an average value for each sample was determined.

Removal torque test
A total of 24 implants (12 per group) were used in this test. The biological specimens were processed immediately after the removal of the tibiae. The samples were maintained in liquid solution (10% buffered formalin) and immediately evaluated (1 h after removal) so as to avoid dehydration. A torque testing machine was used – CME [Técnica Industrial Oswaldo Filizola, Guarulhos, Brazil], which is fully controlled by software DynaView Torque Standard/Pro M (Fig. 2), performing calculations and generating reports automatically with test speed of 1 rpm and angular measuring system with a resolution of 0.002°. Measurements of peak torque to initiate reverse rotation were recorded, and the mean torque values were calculated for each group.

Samples treatment for histomorphometric analysis
The others 24 samples (12 per group) were dehydrated using an ascending series of alcohols and embedded in glycomethacrylate resin (Technovit 9100 VLC; Kulzer, Friedrichsdorf, 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 macrorcutting and grinding system [Isomet 2000; Buehler, Esslingen, Germany]. The sections were stained with toluidine blue and acid fuchsin, and histomorphometric analysis was carried out.

Specimens that had been prepared for the histological analysis of the tissue surrounding the implant were examined using a light microscope [EOS 200; Nikon, Tokyo, Japan]. After digitizing the phase of each specimen under light microscope, the percentage of bone-to-implant contact (BIC%) was measured using the program Image Tool version 5.02 for Microsoft Windows™. BIC% was calculated as the percentage of bone that was in direct contact with the implant surface, evaluated along the entire profile of the implant.

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.

Histological analysis
Histological analysis showed a complete bone organization and mineralization at 8 weeks in both groups [Figs 3 and 4]. The BIC% values are summarized in the Table 3 and did not show statistical differences (P = 0.237). At high magnification, the samples of Con group showed small areas where bone formation has not reached the surface of the implant, probably because of some physical-chemical components that prevented the contact [Fig. 5].

Results
The surgical procedures were uneventful. All animals presented appropriate healing during the first week following the surgical procedure. Post-surgical inspections for 2 weeks postoperatively indicated the absence of infection or inflammation. After the scheduled follow-up time, all implants were osseointegrated.

Resonance Frequency Analysis (RFA)
The data and statistical analysis of resonance frequency values for the times investigated of the two groups are summarized in the Table 1. Applying the test inside the groups at the time period proposed [baseline and 8 weeks], the values showed statistically significant differences (P < 0.05). Among the groups, the variations in the RFA values between the first and the second time point were not significantly different (P > 0.05).

Removal torque test
In removal torque testing, all of the implants were stable and anchored in bone after 8 weeks of healing. The mean resistance to removal torque values and standard deviation are summarized in the Table 2 and were not significantly different (P > 0.05).

Discussion
Over the past decades, a multitude of in vivo studies examined the effect of the implant surface on the bone healing and apposition [Misch 1990, Hsu et al. 2007]. 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 [Textor et al. 2001].

Histology-based investigations have shown that surface texturing created by blasting led to greater bone–implant contact as compared with the machined surface [Ivanoff et al. 2001], 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 [Wennerberg et al. 1996]. Abrasive blasting increases the surface roughness, as well as the metal surface reactivity [Wennerberg et al. 1996]. With the use of a blasting material such as Al2O3, a potential risk of contamination by remnants of blasting particles with dissolution of aluminium ions into the host tissue cannot be excluded [Wennerberg et al. 1996]. It has been reported that Al ions may inhibit normal differentiation of bone marrow stromal cells and normal bone deposition and mineralization [Stea et al. 1992], and aluminium has been shown to induce net calcium efflux from cultured bone [Bushinsky et al. 1995]. 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 [Nimb et al. 1995]. Therefore, other alternative methods were developed to sandblasting to roughening the implant surface, such as the use of resorbable particles based on calcium [Albrektsson & Wennerberg 2004a,b] and particles of TiO2 [Albrektsson & Wennerberg 2004a,b; Buser et al. 2004], 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 have been investigated previously [Gotfredsen et al. 1992; Toni et al. 1994; Wennerberg et al. 1995; Ivanoff et al. 2001; Kohal et al. 2004; Smukler-Monkler et al. 2004; Senerby et al. 2005; Gehrke et al. 2014, 2015]. 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 analysed the specimens using both scanning electron microscopy and histomorphometry, as well as removal torque test in dogs [Gotfredsen et al. 1992]. The present study showed that implants blasted with titanium dioxide particles or aluminium dioxide particles had a good anchorage, with no difference in bone-implant contact.

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 [Hall & Lausmaa 2000]. Thus, either grit blasting alone or in combination with a subsequent acid etching protocol alters not only surface texture but also surface chemistry and wettability, presenting the potential to alter the early interaction between the host biological fluids and implant surface [Ban et al. 2006; Coelho & Lemons 2009]. The application of acid conditioning after the sandblasting using both microparticle media tested on the surface promotes the roundness of the irregularities created, making the surface topography more uniform.

Studies reported that the 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 enhanced by higher surface wetting characteristics [Buser et al. 2004; Yang et al. 2006]. The blasting particle material, either TiO2 or Al2O3, did not show any difference in bone response with respect to removal torque, bone-to-implant contact and bone area after 12-week healing [Wennerberg et al. 1996]. Similar results were found in the present study.

Animal models are essential in providing phenomenological information on biological reaction to implants inserted in bone [Piattelli et al. 1998]. The rabbit represents a common model used in orthopedics [Wennerberg et al. 2003]. This animal model due to its rather fast metabolism and the features of the bone tissue, relatively similar to human bone, provides ideal conditions for the investigation of bone regeneration and implant osseointegration [Lopes & König Júnior 2002; Novaes et al. 2010]. The tibia was chosen as the implant site because of the simplicity of the surgical access [Piattelli et al. 2003]. In the present study, the authors wanted to evaluate the degree of the force of osseointegration and the characteristics of the bone around the surface after 8 weeks. In fact, previous researches 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 [Piattelli et al. 1998]. Histomorphometric and removal torque measurements are two representative tests in studying the nature of the implant tissue interface [Meredith 1998]. Recently, Gehrke et al. 2015 evaluated in vitro a surface SLA where the blasting process of the surface was made using particles of TiO2, and the conclusions were that represent an adequate option for the surface treatment of dental implants, with minimal risk of contamination by the residual debris from the blasting procedure. Another study by Gehrke et al. 2014 demonstrated an excellent

**Table 1. Brunner-Langer test of ISQ measurements and analysis at baseline (initial) day and at 8 weeks. Results as mean and medians were expressed in ISQ values**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Test group</th>
<th>P value (inter-group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISQ Value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>Mean ± SD</td>
<td>Median</td>
<td>71.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>71.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td></td>
<td>0.167</td>
</tr>
<tr>
<td>8 weeks</td>
<td>Mean ± SD</td>
<td>Median</td>
<td>74.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td></td>
<td>0.031*</td>
</tr>
</tbody>
</table>

*Significant differences with P < 0.05.

**Table 2. Descriptive statistics for the outcome variables measured using removal torque measurements**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Test group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>104 ± 6.9</td>
<td>118.9 ± 7.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Range</td>
<td>98–121</td>
<td>104–126</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>103.5</td>
<td>118.9</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Histological pictures showing the bone healing around the implant after 8 weeks. In [left image], Con group showing a little organization and quantity of cells, in [right image], it is possible to observe the greater quantity and the better organization of bone. Magnification: ×4 and ×100, respectively. Picrosirius-haematoxylin staining.
higher bone formation. The reverse torque values were rather high in the samples of the two groups tested, but very similar between them, despite that comparing the average, the Test group presented a removal torque 11.9% higher relative to the Con group, which can be related to the difference in macrodesign of the implants used in this study (Hallgren et al. 2003). 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 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 two different surfaces using both in vitro and in vivo approaches. The results confirm that both blasting media (titanium and aluminium oxide) for surface treatment produced high osteoconductivity and good bone formation.

Conclusion

Within the limitations of this study, the results indicate that the media of surface blasting (AlO2 or TiO2 microparticles) did not show significant differences in the tested parameters for assessing the osseointegration 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.

Table 3. Descriptive statistics for the outcome variables measured of the implant-to-bone contact in percentage (%BIC)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con group</th>
<th>Test group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>65.6 ± 5.7</td>
<td>66.6 ± 4.8</td>
<td>0.237</td>
</tr>
<tr>
<td>Range</td>
<td>55–73</td>
<td>58–73</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>67.0</td>
<td>67.5</td>
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References


Gehrke et al - AlO2 and TiO2 microparticles for surface blasting