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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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 Guéhenneuc et al. 2007]. The macrogeometric design such as the implant body shape, height, density

Key words: aluminium blasted, dental implants, SLA surface, surface treatment, titanium blasted

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.

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and cutting ability of the threads may affect the biomechanics of the bone–implant inter-
locking, possibly improving implant stability (Hallgren et al. 2003).

Several types of chemical and physical sur-
face treatments have been developed and
marketed by dental implant manufacturers
(Binon 2000). However, there is still no con-
sensus on what the optimal condition for
peri-implant bone growth should be. It is
known that the bone response can be influ-
enced 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 biologi-
cal processes involved in the activation of
the early stages of osseointegration, such as
protein adsorption, cell–surface interaction,
progenitor cell recruitment and differentia-
tion and tissue formation at the interface
between the body and the biomaterial, can be
affected by the implant surface microrough-
ness 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 usu-
ally present fine 2–4-μm micropits superim-
posed 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 (Piat-
telli 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; Espos-
ito 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 analy-
ses, the effects of aluminium (AlO2) and tita-
nium dioxide (TiO2) microparticles for
blasting used to produce the SLA surface
Treatmen~ 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 con-
trol group of implants (Con group) with SLA
surface that is produced using the AlO2
microparticles for blasting and subsequent
acids conditioning (Straumann, Basel,
Switzerland) and a test group of implants
(Test group) produced using TiO2 microparti-
cles for blasting and subsequent acid condi-
tioning (Implacril 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 respec-
tive distributors of each product.

Animals and surgical procedure

Eight New Zealand white adult rabbits weigh-
ing 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 experi-
ment was approved by the Ethics Committee
for Animal Research of the University of Mur-
cia (Spain), in accordance with the European
(R.D.53/2013). The rabbits were anaesthetized
with an intramuscular injection of tiletamine/
zolazepam 15 mg/kg (Zoetil 50, Virbac,
Madrid, Spain) and xylazine 5 mg/kg (Rom-
pun; Bayer, Leverkusen, Germany). Before sur-
ery, the shaved skin over the area of the
proximal tibia was washed with Betadine®;
Meda Manufacturing, Burdeos, France. Keta-
mine 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-fely-
pressin, Astra, Mexico) was injected subcuta-
neously at the site of surgery to improve
analgesia and to control bleeding. A skin inci-
sion 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. Postop-
eratively, a single dose of 600,000 IU Ben-
zeotacil 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 postopera-
tive period. All animals were euthanized with
an intravenous overdose of ketamine (2 ml)
and xylazine (1 ml); four animals were killed
each time point: 4 weeks and 6 weeks after
the implantations. Both tibias were removed,
and xylazine (1 ml); four animals were killed
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the implantations. Both tibias were removed,
and xylazine (1 ml); four animals were killed
at each time point: 4 weeks and 6 weeks after
the implantations. Both tibias were removed,
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 macrocutting and grinding system (Isomet 200; 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.

**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 times 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].

**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].

**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
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>Mean (\pm SD)</td>
<td>Median</td>
<td>Mean (\pm SD)</td>
</tr>
<tr>
<td>Control group</td>
<td>71.3 (\pm 1.4)</td>
<td>71</td>
<td>71.4 (\pm 1.7)</td>
</tr>
<tr>
<td>Test group</td>
<td>72.1 (\pm 1.9)</td>
<td>71.5</td>
<td>75.2 (\pm 1.3)</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 (\pm SD)</td>
<td>104 (\pm 6.9)</td>
<td>118.9 (\pm 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>

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; Sennenby 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.

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
Histological pictures showing the bone healing around the implant after 8 weeks. In (left image), Test 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.

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>
<td></td>
</tr>
</tbody>
</table>

Histological pictures showing the cellular proliferation and organization around the implant after 8 weeks in Test group (up image) and Con group (below image). At this high magnification, the samples of Con group showed small areas where bone formation has not reached the surface of the implant (yellow arrows). Magnification: ×400.

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

The biologic response of the surfaces treated by sandblasting with microparticles of titanium oxide followed by acid etching. In this study, both surface biocompatibility and osteoconductive properties were confirmed by the biomechanical tests and the subsequent histological analysis, showing an intimate interaction between newly formed bone and either implant surface of both groups. Such interaction was equally pronounced for both SLA treatment methods, indicating that the biomechanical test results may have been a synergy of the mechanical interlocking between bone and implant surface and the 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.

Fig. 5. Histological pictures showing the cellular proliferation and organization around the implant after 8 weeks. In (left image), Test 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.

Fig. 4. Histological pictures showing the bone healing around the implant after 8 weeks. In (left image), Test 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.

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.

References


