In vivo performance of zirconia and titanium implants: a histomorphometric study in mini pig maxillae

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Abstract

Objectives: To compare the bone tissue response to surface-modified zirconia (ZrO2) and titanium implants.

Methods: Cylindrical low-pressure injection moulded zirconia (ZrO2) implants were produced with an acid-etched surface. Titanium implants with identical shape, sandblasted and acid-etched surface (SLA) served as controls. Eighteen adult miniature pigs received both implant types in the maxilla 6 months after extraction of the canines and incisors. The animals were euthanized after 4, 8 and 12 weeks and 16 zirconia and 18 titanium implants with the surrounding tissue were retrieved, embedded in methylmethacrylate and stained with Giemsa–Eosin. The stained sections were digitized and histomorphometrically analysed with regard to peri-implant bone density (bone volume/total volume) and bone–implant contact (BIC) ratio. Statistical analysis was performed using Mann–Whitney U-test.

Results: Histomorphometrical analysis showed direct osseous integration for both materials. ZrO2 implants revealed mean peri-implant bone density values of 60.4% (SD ± 9.9) at 4 weeks, 65.4% (SD ± 13.8) at 8 weeks, and 63.3% (SD ± 21.5) at 12 weeks after implantation, whereas Ti-SLA implants demonstrated mean values of 61.1% (SD ± 6.2), 63.6% (SD ± 6.8) and 68.2% (SD ± 5.8) at corresponding time intervals. Concerning the BIC ratio, the mean values for ZrO2 ranged between 67.1% (SD ± 21.1) and 70% (SD ± 14.5) and for Ti-SLA between 64.7% (SD ± 9.4) and 83.7% (SD ± 10.3). For the two parameters investigated, no significant differences between both types of implants could be detected at any time point.

Conclusion: The results indicate that there was no difference in osseointegration between ZrO2 implants and Ti-SLA controls regarding peri-implant bone density and BIC ratio.

The patient’s aesthetic demands on dental implants have increased, especially if anterior teeth have to be replaced. From an aesthetic point of view, the grey colour of titanium is disadvantageous and may create problems when becoming visible (Marinello et al. 1997; Glauser et al. 2004; Kohal et al. 2008). Even after successful osseointegration, this might occur due to peri-implant recession formation and concomitant bone resorption. Recently, allergic reactions to titanium have been discussed in this context (Kohal et al. 2009).

Because of the material’s tooth-like colour and due to its biocompatibility, ZrO2 implants have thus become an attractive alternative to titanium (Akagawa et al. 1998; Kohal et al. 2004; Sennherby et al. 2005; Gahlert et al. 2007).

In comparison with other ceramics, ZrO2 exhibit several advantages and is regarded as the most suitable ceramic material for dental implantology (Andreiotelli et al. 2009). Especially, the high fracture toughness and bending strength (Minamizato 1990; Akagawa et al. 1998; Andreiotelli & Kohal 2009; Silva et al. 2009) are rendering the material suitable for oral implantology.

Another advantage of zirconia is the significantly reduced plaque affinity, which reduces the risk of inflammatory changes in the adjacent soft tissue (Scarano et al. 2004). Histological investigations showed that in the peri-implant mucosa inflammatory cells were scarcely seen (Akagawa et al. 1998; Kohal et al. 2004).

Promising results for osseointegration of zirconia implants were demonstrated in several animal studies. Histological studies showed direct osseous integration without any connective tissue formation at the bone–implant interface (Ichikawa et al. 1992; Akagawa et al. 1998; Josset et al. 1999; Schultze-Mosgau et al. 2000; Kohal et al. 2004). Osseointegration was reported under loaded and unloaded conditions in the mandibles and maxillae.
of monkeys. No implant failure has been reported so far [Akagawa et al. 1998; Kohal et al. 2004].

Like in the case of titanium implants, animal studies on zirconia could demonstrate that roughening of the intraosseous implant surface also leads to an increased bone apposition. It was concluded that surface-modified zirconia implants have a comparable capacity for osseointegration as titanium implants [Sennery et al. 2005; Gahlert et al. 2007, 2009; Depprich et al. 2008; Lee et al. 2009; Rocchietta et al. 2009; Koch et al. 2010].

In a recent study, we could demonstrate that 8 and 12 weeks after implantation machined zirconia implants showed statistically significant lower removal torque values than sandblasted ZrO₂ implants and Ti-SLA implants [Gahlert et al. 2007]. In addition, SLA implants showed significantly higher removal torque values than sandblasted zirconia implants after 8 weeks. The findings of a previous histomorphometric study [Gahlert et al. 2009] suggest that low-pressure injection moulded, acid-etched zirconia implants can achieve a comparable functional outcome as conventionally manufactured Ti-SLA implants, even in cases with peri-implant infections.

It was decided to investigate the performance of implants with a new geometrical design using an operative procedure different to that of our previous study [Gahlert et al. 2009].

As in the previous study, direct bone-to-implant contact (BIC) and peri-implant bone density were regarded as key indicators for osseointegration and therefore the aim of the present investigation was to examine these parameters for ZrO₂ implants with a special rough acid-etched surface in comparison with Ti-SLA implants of exactly the same size and design.

Material and methods

Animals

Eighteen female mini pigs [Goettinger mini pig] with an average age of 23.7 months and a weight between 31 and 51 kg were used in this study. The animals were kept in small groups, in cages designed for experimental purposes and fed with a standard diet. Only 12 h before and after surgery, the animals were not given access to food, but had water accessible ad libitum. The protocol of the animal experiment was approved by the Swedish authorities in Malmö (ethical approval number: M 66/07).

Implant design

The implants used in the present study have a shorter thread length [pitch] than the ones investigated previously [Gahlert et al. 2009, Fig. 1] and were implanted without any further use of caps or spacers. Threaded ZrO₂ implants [test group] with a six-cornered shaft, 4.1 mm in diameter and 10 mm in length were manufactured using low-pressure injection moulding technique. After that the implants were chemically treated with hydrofluoric acid, according to a proprietary process of Institut Straumann AG [Basel, Switzerland]. Ti grade 4 implants [control group] with an identical shape were alumina blasted with large grit particles [average particle size 250 μm] and then acid-etched with a hot solution of HCl/ H₂SO₄ [SLA], according to a proprietary process of Institut Straumann AG.

The chemical purity of all surfaces was shown by energy-dispersing X-ray spectroscopy [Philips, Eindhoven, the Netherlands]. Surface topography was qualitatively examined using scanning electron microscopy [Philips] and quantitatively measured by confocal three-dimensional (3D) white light microscopy (μSurf, NanoFocus AG, Oberhausen, Germany) over an area of 770 μm x 770 μm to calculate 3D roughness parameters such as Sₐ [arithmetic mean deviation peak-to-valley height of the surface], Sₚ [maximum peak-to-valley height] and Sₐₖ [amplitude distribution skew] using a Gaussian filter with a cut-off wavelength of 3 μm (Table 1). Surface characteristics of five implants from each group were determined (Table 1).

Table 1. Topographic analyses of the implant surface roughness, values obtained with 3D white light microscopy from an area of 798 x 798 μm; Sₐ = arithmetic mean deviation of the surface roughness, Sₚ = maximum peak-to-valley height found on the surface, Sₐₖ = skewness of the surface

<table>
<thead>
<tr>
<th>Type of implant</th>
<th>Sₐ (μm)</th>
<th>Sₚ (μm)</th>
<th>Sₐₖ (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Median</td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>Ti-SLA (control)</td>
<td>1.26 ± 0.02</td>
<td>1.26</td>
<td>7.62 ± 0.18</td>
</tr>
<tr>
<td>Zirconia (test material)</td>
<td>0.14 ± 0.04</td>
<td>0.14</td>
<td>0.18 ± 0.04</td>
</tr>
</tbody>
</table>

Mean values (± standard deviation (SD)) and medians from five implants, each implant tested at six different positions between second and third thread.

Study design

The study was performed in two surgical phases. In the first phase, the first to third teeth of the maxilla were removed bilaterally in all pigs. After 6 months of healing, the created edentulous areas of the maxilla were exposed by the elevation of buccal mucoperiostal flaps following implant site preparation (second surgical phase).

At any given time point (4, 8 and 12 weeks), the same number of ZrO₂ (test) and Ti-SLA (control) implants was placed at corresponding positions in the front teeth position. Thus, every pig received two implants in the anterior regions of the maxilla to gain comparable donor site behaviour. Finally, a total of 36 implants (18 zirconia and 18 titanium implants) were placed. The study design allowed two implants of the same type in two of the six animals per group.

The implants were allowed to heal submerged. No cap or other spacer device was used to prevent soft or hard tissue overgrowth. The animals were divided into three groups of six animals and sacrificed after 4, 8 and 12 weeks of healing.

Surgical procedures

The animals were anaesthetized by intramuscular (i.m.) injection of ketamine [Ketalar Vet,
in 4% buffered paraformaldehyde and stored at
implants were obtained. All specimens were fixed
were dissected and specimens containing the
ficed with an overdose of pentobarbital (Narcorens,
Merial GmbH, Hallbermoos, Germany). The jaws
were used as analgesia by local injection. The
animals also received an antibiotic treatment with penicillin (Streptocillin® vet. 250 mg, Boehringer Ingelheim, Germany) that was started 1
day before the tooth extraction/surgical proce-
dures and was continued for 7 consecutive days.

During the first surgical procedure, the first to
tooth of the maxilla were removed, the
resulting defects filled with collagen sponges
(Tissuecone/E collagen B2433, Baxter, Deer-
field, IL, USA) and covered with extended
mucoperiostal flaps to provide sufficient healing
conditions. If necessary (e.g. fracture of teeth dur-
ing extraction), careful osteotomy was conducted.
The mucoperiostal flaps were repositioned and
sutured with non-resorbable interrupted sutures.

The second surgery was performed 6 months
later. The recipient sites in the created edentu-
lous regions of the maxilla were exposed by the
elevation of buccal mucoperiostal flaps. If neces-
sary, the alveolar crest was flattened to allow for
fine preparation of the implant recipient sites,
using precise spiral drills with an increasing
size at 500 r.p.m. and copious irrigation
with sterile physiological saline. Subsequently,
the thread was cut into the bone cavity with a
tap. Both types of implants were inserted on
either side of the maxilla and the peak torque
value during implantation was measured. To
reduce early loading of the inserted implants
during the healing period, the contralateral
canines of the mandible were ground.

Animal sacrifice and retrieval of specimens
The animals were anaesthetized by i.m. injection of
ketamine (Ketalar Vet, 50 mg/ml, Pfizer) and mid-
azolam (Dormicum®, 5 mg/ml, Roche) and sacri-
fied with an overdose of pentobarbital (Narcorens,
Merial GmbH, Hallbergmoos, Germany). The jaws
were dissected and specimens containing the
implants were obtained. All specimens were fixed
in 4% buffered paraformaldehyde and stored at
room temperature for 10–15 days.

Histological preparation
The specimens were rinsed in water to wash out
the paraformaldehyde, dehydrated in ascending
alcohol fractions (50%, 70%, 96%, 100%), defor-
ted in xylene and embedded in methylnecryl-
late (Fluka, Switzerland). Serial sections with an
initial thickness of 200 μm were obtained in a
buco-palatal plane using a saw-microtome (Leica
SP 1600, Leica, Wetzlar, Germany). Contact
radiographs (Faxitron X-ray Corporation, Lin-
colnhire, IL, USA) with Agfa Strukturix X-ray
sensitive film (Agfa-Gevaert, Mortsel, Belgium)
were taken from each section before further pro-
cessing. Based on contact radiographic evaluation
of all slides sections representing a longitudinal
cut through the centre of the implant (i.e. aligned
to its long axis) were selected and glued on plastic
slides, ground, polished and surface stained with
Giemsa–Eosin. The final thickness of the stained
sections was 120 ± 20 μm.

Histomorphometrical analysis
For histomorphometrical analysis, sections repre-
senting a cut through the centre of the implant
(i.e. plane of section aligned to long axis of the
implant) were selected. This ensured that
no overlap between implant material and neigh-
boring bone tissue would interfere with the
accuracy of the measurements. The outcome
parameters for the histomorphometric analysis
were the amount of bone (i.e. bone volume [BV]/
total volume [TV]) in a 1 mm region of interest
(ROI) around the implant and the BIC. Initially,
the stained sections were digitized using a
digital camera (Axiocam HRc, Zeiss) attached
to a macroscope (MacroFluo, Leica, Glattbrugg,
Switzerland) in the transparency mode. The
ROI was manually defined, starting at the first
and ending at the last thread of the implant
using Zeiss Axiovision® 4.5 software. Finally,
the threshold interval for mineralized bone
was manually defined and the sections were
analysed using Zeiss KS400 image analysis
software in the semiautomatic segmentation
mode. The bone density value is expressed as
percent bone area within the ROI (BV/TV, Parfitt
et al. 1987; Tonino et al. 1999). The BIC ratio
represents the amount of bone in direct contact to
the implant surface within the ROI (Tonino et al.
1999, Fig. 2).

Fig. 2. (a) Example of a region in which the bone within a maximal distance of 1 mm from the implant surface was assessed
(yellow rectangle), scale bars = 1 mm. (b) The bone density value is expressed as percent bone area within the region
of interest. In this pseudocolour image, the bone area is marked by red colour. (c) Example of bone–implant contact (BIC) rate
assessment. The red line represents BIC, the blue line represents no contact. The ratio gives the amount of BIC (in %) of the
whole length of the red and blue line.
Statistical analysis
Statistical analysis was performed using SPSS PC Version 18 (IBM Deutschland GmbH, Munich, Germany). Median, mean and standard deviations were calculated for each parameter and type of implant for all three time points. Statistical analysis of the data was performed using the Mann–Whitney U-test. P-values < 0.05 were considered to be significant. Scatter plots were obtained to show the distribution of the values.

Results
One animal died immediately after the operation and the implants were excluded from this study. The other animals were sacrificed 4, 8 and 12 weeks after implantation and the resulting group size thus were six, six and five animals. During the postoperative healing period, no dehiscence of the gingiva covering the implants was observed. Clinical inspection did not reveal any signs of inflammation.

Histology and histomorphometry
After 4–12 weeks of implantation, test (ZrO2) and control (Ti-SLA) implants showed direct osseous integration and presented values between 60.4% and 68.2% for peri-implant bone density and between 64.7% and 83.7% for BIC ratio (Tables 2 and 3, Fig. 3).

At all time points, the histomorphometrical data obtained for the entire implant thread length did not reveal statistically significant differences between test (ZrO2) and control (Ti-SLA) implants for peri-implant bone density and BIC ratio (Tables 2 and 3, Figs 4 and 5).

With two exceptions, the mean peri-implant bone density and BIC values of the control implants were always higher than those of the tested ZrO2 implants (Tables 2 and 3). In the 8-week group, the Ti-SLA implants exhibited a slightly lower value for peri-implant bone density and in the 4-week group for BIC ratio (Tables 2 and 3).

For both types of implants, osseointegration values for the entire implant length were reduced when the implant protruded into the nasal cavity or sinus maxillaries. In such cases, a non-osseous, connective tissue interface formed around the implant.

Discussion
The purpose of the present study was to investigate the direct BIC ratio and peri-implant bone density for ZrO2 implants with a rough acid-etched surface topography in comparison with equally shaped Ti-SLA implants in the maxilla of pigs. In a previous investigation, implants with an altered shape were implanted using an operation procedure and postoperative treatment, which were different from the set-up of the present investigation. The previous investigation presented relatively low absolute values for the two parameters investigated (Gahlert et al. 2009). In the present study, the newly designed implants after 4, 8 and 12 weeks of bone healing showed no statistically significant difference between the HF-treated ZrO2 implants and the SLA titanium implants for peri-implant bone density or BIC ratio. The values obtained in this study generally were higher than the values from our previous study (Gahlert et al. 2009) and were comparable to values reported from other groups (Buser et al. 1991). A reason for the magnitude of the values observed here may be related to the fact that the peri-implant inflammation rate was much lower in the present study than in the previous study.
Fig. 4. Scatter plot with the peri-implant bone density values of titanium [Ti-SLA] and zirconia [ZrO₂] implants. Although the median values for zirconia implants were always higher than those of the titanium implants, there was no statistically significant difference between groups [Mann-Whitney U-test, 4 weeks: P = 0.873, 8 weeks: P = 0.631, 12 weeks: P = 0.831].

Fig. 5. Scatter plot for bone-implant contact [BIC] ratio values of titanium [Ti-SLA] and zirconia [ZrO₂] implants. There was no statistically significant difference between groups [Mann-Whitney U-test, 4 weeks: P = 0.337, 8 weeks: P = 0.15, 12 weeks: P = 0.286].

References


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The postoperative treatment in the present study did not rely on the initial use of caps to prevent tissue overgrowth over the implant shaft. Instead, a simple closure of the gingival injury was achieved and this probably explained the lack of inflammatory signs observed during the postoperative healing period. The latter observation could also be related to the fact that the canines of the mandible were ground and thus the probability of early undesired mechanical irritation of the healing zone was reduced.

A second reason may be related to the implant design itself. The first thread of the implants used in the present study is situated deeper in the bone than it was the case in the previously used implant design. This certainly results in a much lower probability that peri-implant infections may interfere with the bone parameters that are measured during histomorphometry.

The BIC values measured in the present study are comparable to data reported in the literature by Buser et al. (2004) and are higher than those observed in other studies (Gahlert et al. 2009; Rocchietta et al. 2009).

In our study, most implants from both groups histologically showed an excellent osseous integration and this is reflected in the magnitude of the peri-implant bone density values. This again may be related to the creation of submerged and thus undisturbed healing conditions.

The results of the present study clearly show that in an experimental pig model there was no difference in osseointegration detectable between the two types of implants. Because of the limited number of samples, a direct extrapolation of the results to the situation in the human patient was not possible.

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