Osseointegration of zirconia and titanium dental implants: a histological and histomorphometrical study in the maxilla of pigs

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Abstract
Objectives: The purpose of the present study was to histologically compare the bone tissue responses to surface-modified zirconia and titanium implants.

Methods: Threaded zirconia implants were produced using a new low-pressure injection moulding technique and thereafter surface treated by acid etching. Titanium implants with the exact shape and surface treated by sandblasting and acid etching (SLA) served as controls. Fifteen adult pigs received both implant types in the maxilla 6 months after extraction of the second and third incisors. The animals were sacrificed after 4, 8 and 12 weeks and 30 implants with surrounding bone were retrieved.

Results: Histological evaluation showed osseous integration for both materials. Zirconia implants revealed mean peri-implant bone density values of 42.3\% (SD ± 14.5) at 4 weeks, 52.6\% (SD ± 5.7) at 8 weeks and 54.6\% (SD ± 11.5) at 12 weeks after implantation, whereas Ti-SLA implants demonstrated mean values of 29\% (SD ± 10), 44.1\% (SD ± 18) and 51.6\% (SD ± 8.6) at corresponding time intervals. With respect to the bone–implant contact ratio, the mean values for zirconia ranged between 27.1\% (SD ± 3.5) and 51.1\% (SD ± 12.4) and for Ti-SLA, it ranged between 23.5\% (SD ± 7.5) and 58.5\% (SD ± 11.4).

For the parameters investigated, no statistically significant differences between both types of implants could be detected at any time point.

Conclusions: No statistical difference between implants could be demonstrated with any of the methods used. The limited number of animals per group, however, does not allow to conclude that there is no difference in osseointegration between the two types of implants, although the data tend to suggest such a trend.

Tooth replacement with dental implants in partially and completely edentulous patients has become a well-documented and scientifically accepted treatment modality. The implant material used most often is titanium (Steinemann 1998; Albrektsson et al. 2008). A disadvantage from an aesthetic point of view is the grey colour of titanium, which may create a problem in cases of visible titanium or thin soft tissue coverage (Marinello et al. 1997; Glauser et al. 2004; Kohal et al. 2008). Furthermore, due to peri-implant recesses, which often coincide with bone resorption, the implant head might become visible over time, even when it was initially well covered by bone and soft tissue. One approach to overcome this problem is to use an implant material with a different, more natural colour. Zirconia implants have thus become an attractive alternative to titanium because of the material’s...

Zirconia ceramics, in comparison with other ceramics, exhibit several advantages, the most important being their high fracture toughness and bending strength (Minamizato 1990; Akagawa et al. 1998). Animal studies have demonstrated promising results for osseous integration of zirconia implants under both unloaded and loaded conditions in the mandibles of eight and maxillae of six monkeys, respectively, and no implant failure was ever reported (Akagawa et al. 1998; Kohal et al. 2004). In addition, histological investigations showed high degrees of bone-implant contact (BIC) (Ichikawa et al. 1992; Akagawa et al. 1998; Josset et al. 1999; Schultze-Mosgau et al. 2000; Kohal et al. 2004; Sennerby et al. 2005; Gahlert et al. 2007). Soft tissue response was also regarded as good (Akagawa et al. 1998; Kohal et al. 2004). Another advantage of zirconia is the significantly reduced plaque affinity, which reduces the risk of inflammatory changes in the adjacent soft tissue (Akagawa et al. 1998). Taken together, the available data indicate that zirconia ceramics is a material suitable for dental implant manufacturing (Akagawa et al. 1998; Kohal et al. 2004; Sennerby et al. 2005; Gahlert et al. 2007). However, there are only two clinical case reports and two retrospective observational case series published using zirconia implants (Kohal and Klaus 2004; Volz and Blaschke 2004; Blaschke and Volz 2006; Oliva et al. 2007), and only survival rates were reported. Moreover, in a recent review on zirconia abutments and implants, the scientific support for the clinical use of zirconia implants was regarded as very low (Kohal et al. 2008). There are different approaches to roughening zirconia implant surfaces like sandblasting (Kohal et al. 2004; Gahlert et al. 2007) or coating with zirconia powder (Sennerby et al. 2005). In a recently published study, we could demonstrate that after 8 and 12 weeks of implant placement, machined zirconia implants showed statistically significant lower removal torque values (RTV) than sandblasted ZrO2 implants and Ti-sandblasting and acid etching (SLA) implants. In addition, SLA implants showed significantly higher RTVs than sandblasted zirconia implants after 8 weeks. These findings suggest that sandblasted zirconia implants can achieve a higher mechanical stability in bone than conventionally machined zirconia implants. Roughening the surface of machined zirconia implants enhances bone apposition and enhances the ability to withstand shear stress (Gahlert et al. 2007).

As direct BIC and peri-implant bone density are regarded as key indicators for successful osseointegration, the aim of the present study was to investigate these parameters for zirconia implants with a new rough acid-etched surface topography in comparison with Ti-SLA implants.

Material and methods

Animals
Fifteen pigs of an average age of 18 months and a weight between 60 and 110 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 study was designed according to § 8 of the German law against cruelty to animals and was approved by the local authorities [reference: Regierung von Oberbayern AZ 55.2-1-54-2531-62-06].

Implant design
Zirconia implants with a tapered effect (TE) thread and a four-cornered shaft, 4.1 mm in diameter and 10 mm in length, were manufactured using the low-pressure injection moulding technique. Subsequently, the implants were chemically treated with a hot solution of HCl/H2SO4 according to a proprietary process of Institut Straumann AG. The chemical purity of all surfaces was proven by energy-dispersive X-ray spectroscopy (EDX, Philips, Eindhoven, the Netherlands) at different magnifications. Surface topography was qualitatively examined using scanning electron microscopy (SEM, Philips) and quantitatively measured by confocal 3D white light microscopy (µsurf, NanoFocus AG, Oberhausen, Germany) over an area of \(770 \mu m \times 770 \mu m\) to calculate three-dimensional roughness parameters such as \(S_m\) [arithmetic mean deviation peak-to-valley height of the surface], \(S_t\) [maximum peak-to-valley height] and \(S_{sk}\) [amplitude distribution skew] using a Gaussian filter with a cut-off wavelength of 31 \(\mu m\) (Table 1).

Study design
The study was performed in two surgical phases. In the first phase, the second and third incisors of the maxilla were removed in all pigs. After 6 months of healing, the created edentulous areas of the maxilla were exposed by the elevation of buccal mucoperiosteal flaps following implant site preparation. Subsequently, one zirconia and one Ti-SLA implant per pig were placed according to a split-mouth design using a randomized scheme. The implants were allowed to heal submerged. A total of 30 implants (15 zirconia and 15 Ti-SLA implants) were placed according to this scheme.

The animals were sacrificed after 4, 8 and 12 weeks of healing, yielding groups of 4, 6 and 5 animals.

Surgical procedures
The animals were sedated by an intramuscular (i.m.) injection of Ketamin (10–15 mg/kg), Azaperon (2 mg/kg) and Atropine sulphate (0.5 mg/animal). Anaesthesia was induced with an intravenous bolus

| Table 1. Topographic analyses of the implant surface roughness |
|---------------------------------|---------|---------|---------|
| Type of implant (n = 5)         | \(S_m\) (\(\mu m\)) | \(S_t\) (\(\mu m\)) | \(S_{sk}\) (\(\mu m\)) |
| Ti-SLA                          | 1.17 ± 0.04 | 7.75 ± 0.55 | 4.25 ± 2.31 |
| Zirconia                        | 0.55 ± 0.01 | 3.71 ± 0.12 | −0.15 ± 0.06 |

\(S_m\) arithmetic mean deviation of the surface roughness; \(S_t\) maximum peak-to-valley height found on the surface; \(S_{sk}\), amplitude distribution skew; SLA, sandblasting and acid etching.
of Propofol 1% (5 ml), followed by incubation and maintenance of anaesthesia by inhalation of 1.5% Isoflurane and 2% Propofol. For general analgesia, the animals received a first injection of Caprofen [4 mg/kg] i.m. 24 h before surgery and a premedication with Metamizol [40 mg/kg], solved in Ringers solution. Additionally, 2% Lidocaine [8–15 ml] was used as analgesia by a local injection. For postsurgical treatment, an injection of Enrofloxacin [2.5 mg/kg] was administered i.m for 2 days.

During the first surgical procedure, the second and third incisors of the maxilla were removed under general anaesthesia using extended mucoperiostal flaps to provide sufficient access to the alveolar crest containing the teeth to be removed. If necessary, careful osteotomy was conducted. Following teeth removal, the elevated flaps were repositioned and sutured with non-resorbable interrupted sutures.

The second surgery was performed after 6 months of healing. The recipient sites in the created edentulous areas of the maxilla were exposed by the elevation of buccal mucoperiostal flaps. When necessary, the alveolar crest was flattened to allow for precise preparation of the implant recipient sites, using precise spiral drills of increasing diameter at 500 rpm and copious irrigation with sterile physiological saline. Subsequently, the thread was cut into the bone cavity with a tap. One zirconia implant and one Ti-SLA implant were inserted on each side of the maxilla of each pig and left to heal in a submerged position. Thus, primary stability with the bone walls of the implant beds was achieved by the press-fit design and the screw thread.

**Animal sacrifice and retrieval of specimens**

The animals were sacrificed with an overdose of Pentobarbital and potassium chloride, the jaws were dissected and bone blocks containing 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 remove the paraformaldehyde, dehydrated in ascending alcohol fractions (50%, 70%, 96% and 100%), defatted in Xylene and embedded in methylmethacrylate (MMA, Fluka, Switzerland). Serial sections with an initial thickness of 200 μm were obtained in a buco palatinal plane using a saw-microtome (Leica SP 1600, Leitz, Germany). Contact radiographs [Faxitron [Faxitron X-Ray LLC, Lincolnshire, Illinois 60069, USA] with an Agfa Strukturix X-ray sensitive film (Agfa, Agfa-Gevaert AG, Dübendorf, Switzerland) were taken from each section before further processing. Based on contact radiographic evaluation of all slides, sections representing a cut through the centre of the implant (along its long axis) were selected and glued on plastic slides, ground, polished and stained with toluidine blue. The final thickness of the stained sections was 120 ± 20 μm.

**Histological analysis**

Analysis of the samples was performed using an Axioplan 2 microscope (Zeiss, Carl Zeiss GmbH, Göttingen, Germany) equipped with Plan-Neofluar objectives [× 5, × 10]. Microscopic images were obtained with an Axiocam digital camera (Zeiss). For histological evaluation a qualitative (i.e. descriptive) scheme was used to classify the results. The evaluation mainly focused on the presence/absence of inflammatory signs, type of surrounding tissues (e.g. bone, dentine fibrous connective tissue) and the topographic relation of these tissues to the implant.

**Histomorphometrical analysis**

For histomorphometric analysis, contact radiographs of the sections representing a cut through the centre of the implant (along its long axis) were selected. This ensured that no overlap between implant material and neighbouring bone tissue would interfere with the accuracy of the measurements. The radiographs were digitized using a digital camera with a macroscope (MacroFluo, Leica) in the transparency mode. The outcome parameters for the histomorphometric analysis of the contact radiographs were the amount of bone (i.e. bone volume/total volume) in a 1 mm region of interest (ROI) around the implant and the BIC ratio (Fig. 1a,b). The ROI was manually defined, started at the first and ended at the last thread, and corresponds to the entire inserted length of the implant. To evaluate the influence of crestal resorption a second ROI definition was used. In this case the ROI started at the point of first BIC and ended at the last thread.

The measurements were obtained by combined use of Zeiss Axiovision® 4.5 and Zeiss KS400 image analysis software (Zeiss). During this process, KS400 image analysis software was used to perform the automatic segmentation (i.e. recognition) of mineralized bone tissue.

**Statistical analysis**

Statistical analysis was performed using a commercially available software program (SigmaStat, Systat Software Inc., USA). Mean values and standard deviations were calculated for each implant in each pig. Statistical analysis of the data was performed using Student’s t-test if values passed the test for normality (i.e. were likely to exhibit a normal distribution). If the values were not...
normally distributed or the Equal Variance Test failed, the Mann–Whitney rank sum test was used. *P*-values < 0.05 were considered to be significant.

Results

The SEM micrographs showed a similar microstructure for the zirconia implant surface [Fig. 2a] compared with the Ti-SLA surface [Fig. 2b]. However, zirconia seemed to have a flatter profile with less porosity in comparison with the SLA surface, resulting in a less rough macrostructure. EDX analysis indicated pure ZrO₂ implant surfaces. SLA surfaces were characterized by a Ti oxide surface. Ti-SLA implants with an \( S_a \) value of 1.17 ± 0.04 μm are about twice as rough as zirconia implants with an \( S_a \) value of 0.55 ± 0.01 μm (Table 1).

**Histology and histomorphometry**

One zirconia and one Ti-SLA implant were lost due to insufficient integration. These two implants were consequently excluded from further analysis. The resulting implant numbers per group and implant type were four [4 weeks], five [8 weeks] and five [12 weeks]. The other implants showed osseointegration and presented higher values for peri-implant bone density and BIC ratio (Tables 2 and 3; Fig. 3a,b).

The histomorphometrical data obtained for the entire implant length did not reveal any statistically significant difference between both types of implants for peri-implant bone density and BIC ratio. This observation was the same at all time points investigated (Tables 2 and 3). In both groups of implants the bone density values showed an increase from the fourth to the 12th week.

Statistically significant differences in the BIC ratio were only observed between the values of the early phase of bony integration [4-week group] and the later phases [12 weeks, and in one case, the 8-week group]. Peri-implant bone density only showed significant differences between weeks 4 and 12 around titanium implants but not around zirconium dioxide implants.

Within the same animal, the peri-implant bone density values for titanium and zirconium dioxide implants exhibited a lower variation than the values of the entire group. In individual animals, the zirconium dioxide implants exhibited markedly better peri-implant bone density values than the titanium implants but this was not statistically significant for the whole group. The median peri-implant bone density values of the zirconium dioxide implants were always higher than those of the titanium implant group (Fig. 4).

In contrast to the values for bone density, the BIC ratio values exhibited a greater variation for both types of implants. However, within the same animal, the variation was again lower than the variation for the entire group. In some animals the titanium implants exhibited markedly better BIC ratio values than the zirconium dioxide implants but there was no statistically significant difference between the two groups. For weeks 8 and 12, the median BIC ratio values of the titanium implants were slightly higher than the values of the zirconium dioxide implant group (Fig. 5). This was not yet observed in week 4.

**Table 2. Peri-implant bone density values (percent, mean ± SD) for Ti-SLA and zirconia implants**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Ti-SLA</th>
<th></th>
<th>Zirconia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First to last thread</td>
<td>First bone-implant contact to last thread</td>
<td>First to last thread</td>
</tr>
<tr>
<td>4</td>
<td>29 ± 10.8</td>
<td>55.1 ± 8.6</td>
<td>42.3 ± 14.5</td>
</tr>
<tr>
<td>8</td>
<td>44.1 ± 18</td>
<td>70.4 ± 15.6</td>
<td>52.6 ± 5.7</td>
</tr>
<tr>
<td>12</td>
<td>51.6 ± 8.6</td>
<td>54.4 ± 13</td>
<td>54.6 ± 11.5</td>
</tr>
</tbody>
</table>

The values for the entire implant (region of interest defined from first to last thread) and parts with direct bone contact (region of interest reaching from first bone-implant contact to the last thread) are given.

*Significant difference in comparison with the 4-week Ti-SLA group (*P* = 0.001).

**Table 3. Bone–implant contact (BIC) ratio values (percent, mean ± SD) for Ti-SLA and zirconia implants**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Ti-SLA</th>
<th></th>
<th>Zirconia</th>
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<tr>
<td></td>
<td>First to last thread</td>
<td>First bone-implant contact to last thread</td>
<td>First to last thread</td>
</tr>
<tr>
<td>4</td>
<td>23.5 ± 7.5</td>
<td>44.2 ± 7.9</td>
<td>27.1 ± 3.5</td>
</tr>
<tr>
<td>8</td>
<td>53.3 ± 27.6</td>
<td>83.3 ± 6.3</td>
<td>51.9 ± 14*</td>
</tr>
<tr>
<td>12</td>
<td>58.5 ± 11.4*</td>
<td>64.7 ± 15.8</td>
<td>51.1 ± 12.4*</td>
</tr>
</tbody>
</table>

The values for the entire implant (region of interest defined from first to last thread) and parts with direct bone contact (region of interest reaching from first bone-implant contact to the last thread) are given.

*Significant difference in comparison with the 4-week zirconia group (*P* = 0.032).
*Significant difference in comparison with the 4-week zirconia group (*P* = 0.008).
*Significant difference in comparison with the 4-week Ti-SLA group (*P* = 0.01).

SLA, sandblasting and acid etching.

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Fig. 2. Representative scanning electron microscopic micrograph of the (a) zirconia surface and (b) Ti-SLA sandblasting and acid etching surface. Note the comparable surface topography of the two materials [scale bars: 20 μm].

**Table 1. Microstructure for the zirconia implant surfaces.**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Microstructure for the zirconia implant surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>SLA, sandblasting and acid etching.</td>
</tr>
<tr>
<td>8</td>
<td>SLA, sandblasting and acid etching.</td>
</tr>
<tr>
<td>12</td>
<td>SLA, sandblasting and acid etching.</td>
</tr>
</tbody>
</table>

*Significant difference in comparison with the 4-week zirconia group (*P* = 0.001).
For both types of implants, osseointegration values for the entire implant length were low when the implant protruded out of the bone and had contact with the oral cavity. The values for peri-implant bone density and BIC increased when the second ROI definition (first BIC to the last thread) was used.

The non-osseous interface was formed by loose or dense connective tissue, which, in a few cases, showed mononuclear cell enrichment. In such cases, a diagnosis of ‘inflammatory reaction’ was made. Non-inflammatory inhibition of osseointegration occurred when the implant was positioned to some extent into the remaining incisor, the respective periodontal ligament or into the nearby palatinal suture. In all these cases, a non-osseous, connective tissue interface formed between the implant and the mineralized tissue (Fig. 3c,d).

Discussion

To date, only a limited number of histological and histomorphometrical studies have been performed using zirconia implants. Akagawa et al. (1998) examined the possibility of the long-term stability of osseointegration around partially stabilized zirconia implants with a 1-stage procedure with different loading designs in the mandibles of eight monkeys. They did not observe a clear difference in the clinical features among the different types of support. Direct bone apposition to the implant was generally seen in all the groups. The bone contact ratio ranged from 66% to 81%, and the bone area ratio varied between 49% and 78% at 24 months after loading. Kohal et al. (2004) investigated the osseointegration of loaded zirconia implants in comparison with titanium implants in the maxilla of six monkeys. The titanium implant surfaces were sandblasted with Al₂O₃ and subsequently acid-etched. The zirconia implants were only sandblasted. The mean BIC after 9 months of healing and 5 months of loading was 67.4% (SD: 17%) for the zirconia implants and 72.9% for the Ti-SLA implants. No statistically significant difference between the different implant materials was detected. Gahlert et al. (2007) investigated zirconia implants with either a machined or a sandblasted surface and compared them with sandblasted and acid-etched Ti-SLA implant surfaces in the maxilla of minipigs. The machined ZrO₂ implants showed statistically significant lower RTQ values than the other two implants types after 8 and 12 weeks. The authors concluded that roughening the machined zirconia implants enhances bone apposition and has a beneficial effect on the interfacial shear strength. In addition, 13 implants were evaluated histologically for the apposition of mineralized structures.
The purpose of the present study was to investigate the direct BIC ratio and peri-implant bone density for zirconia implants with a new rough acid-etched surface topology in comparison with Ti-SLA implants in the maxilla of pigs. After 4, 8 and 12 weeks of bone healing, the results showed no statistically significant difference between the HF-treated ZrO2 implants and the SLA titanium implants at any time point for peri-implant bone density or BIC ratio. The peri-implant bone density values for zirconia increased from 42.3% after 4 weeks to 54.6% after 12 weeks whereas the values for Ti-SLA increased from 29% to 51.6%, respectively. In terms of the BIC ratio, the values increased from 27.1% to 51.1% for the zirconia implants and from 23.5% to 58.5% for the Ti-SLA implants, respectively. The measured BIC values for Ti-SLA are in the same range as the values measured in the tibia and femur of mini-pigs by Buser et al. (1991). They reported BIC values of 52.1% after 3 weeks and 57.7% after 6 weeks. In another study, the same group reported BIC values of 66.6% and 75.5% after 4 and 8 weeks, respectively, in the maxilla of minipigs (Buser et al. 2004), which are higher than those observed in the present study. This difference may be related to the specially designed bone chamber implants Buser et al. (2004) used. Another interpretation could be that different definitions of the ROI contribute to the high variability of values given in the literature. Comparing our own results obtained from the same implants but with two different definitions of the ROI, it becomes obvious that the values vary significantly. We think that only the values obtained from the entire implant may characterize the clinically relevant performance. The second ROI definition (first BIC to the last thread) neglects zones with apical bone resorption and the values obtained do not reflect the performance of the entire implant; these values are potentially misleading and therefore should not be taken into account when comparing the two types of implants.

In our study, most implants from both groups histologically showed a very good osseous integration. However, a small number of implants (from both groups) showed some problems regarding bony integration with or without an inflammatory reaction in the vicinity of the implant. Osseous integration in the non-inflammatory cases was limited either when an implantation in or close to a growing or a potentially moving structure occurred. This was the case when the implant was in contact with the remaining incisor or the palatal suture. In cases where the implantation was performed in purely osseous host tissue, these problems were absent and osseointegration was good.

The overall result that equivalent osseointegration values were found for zirconia implants in comparison with titanium implants is in line with the findings of Akagawa et al. (1998) and Kohal et al. (2009), who demonstrated the beneficial effect of roughened zirconia implants.

The present results show that for osseointegration in an experimental pig model, no statistically significant difference was detectable between the two types of implants. However, since the results are based on a limited number of samples, the conclusion that there is no difference cannot be drawn, although the data tend to suggest such a trend. Based on this consideration, a direct extrapolation of the results to the outcome in the human patient is not possible.

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