Evaluation of a New Titanium-Zirconium Dental Implant: A Biomechanical and Histological Comparative Study in the Mini Pig

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ABSTRACT

Background: Titanium zirconium alloy with 13–17% zirconium (TiZr1317) shows significantly better mechanical attributes than pure Ti with respect to elongation and fatigue strength. This material may be suitable for thin implants and implant components exposed to high mechanical constraints.

Purpose: The aim of this study was to test the hypothesis that TiZr1317 and Ti implants show comparable osseointegration and stability.

Materials and Methods: The mandibular premolars (P1, P2, P3) and the first molar (M1) in 12 adult miniature pigs were extracted 3 months prior to the study. Six specially designed implants made from Ti (commercially pure, Grade 4) or TiZr1317 (Roxolid®, Institut Straumann AG, Basel, Switzerland) with a hydrophilic sandblasted and acid-etched (SLActive, Institut Straumann AG, Basel, Switzerland) surface were placed in each mandible; three standard implants modified for evaluation of removal torque (RT) in one side and three bone-chamber implants for histologic observations in the contralateral side. RT tests were performed after 4 weeks when also the bone chamber implants and surrounding tissue were biopsied for histologic analyses in ground sections.

Results: The RT results indicated significantly higher stability ($p = 0.013$) for TiZr1317 (230.9 ± 22.4 Ncm) than for Ti implants (204.7 ± 24.0 Ncm). The histology showed similar osteoconductive properties for both implant types. Histomorphometric measurements showed a statistically significant higher ($p = 0.023$) bone area within the chamber for the TiZr1317 implants (45.5 ± 13.2%) than did the Ti implants (40.2 ± 15.2%). No difference was observed concerning the bone to implant contact between the groups with 72.3 ± 20.5% for Ti and 70.2 ± 17.3% for TiZr1317 implants.

Conclusion: It is concluded that the TiZr1317 implant with a hydrophilic sandblasted and acid-etched surface showed similar or even stronger bone tissue responses than the Ti control implant

KEY WORDS: experimental study, histology, pig mandible, removal torque, TiZr1317

INTRODUCTION

Titanium has long since been the material of choice for dental implants because of favorable physical and chemical properties and its ability to integrate with bone.[1] Titanium is a relatively soft material which may present fatigue problems when used in thin implants and in implant components exposed to high constraints. The strength of titanium can be increased by alloying with other metals. For instance, titanium zirconium alloys with 13–17% zirconium (TiZr1317) shows significantly better mechanical attributes, such as increased elongation and the fatigue strength, than pure titanium.[2]

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Various techniques such as blasting, etching, oxidation, or combination of techniques can be used to increase the surface roughness of dental implants, which have been shown to improve the integration process.[3] Numerous studies have shown that bone is formed directly on rough surfaces, while smoother surfaces seem to be integrated by ingrowth of bone from the vicinity.[4–7] One plausible explanation is that the rough surface retains the blood clot better than smooth surfaces, and that primitive cells can use the fibrin network for migration to the implant surface and subsequent differentiation to osteoblasts. The biomechanical consequence of this is that the rougher implant shows a high resistance to reverse torque at an earlier stage than smooth control implants. One such surface is the SLA (*sand-blasted*, *large-grit*, acid-etched) (Institut Straumann AG, Basel, Switzerland) surface which is produced by sandblasting and acid-etching[8] and has recently been further developed by increasing the hydrophilicity (SLActive, Institut Straumann AG, Basel, Switzerland).[9–11] Recent investigations have demonstrated difficulties in producing identical hydrophilic SLA surfaces on Ti and common Ti alloys ($\alpha + \beta$ biphasic metal structure) because of different responses to etching of the two phases. However, TiZr1317 is an alloy with a monophasic α-structure where sandblasting and acid etching results in a topographically identical surface as on pure titanium implants.[2]

The aim of this study was to test the hypothesis that TiZr1317 and Ti implants with identical hydrophilic sandblasted and acid-etched surface show a comparable osseointegration and stability, as investigated by histomorphometric and biomechanical (removal torque (RT)) measurements after 4 weeks of healing in the mandible of mini pigs.

MATERIAL AND METHODS

Animals and Anesthesia

The study was approved by the ethics committee for animal research at Malmö University, Sweden. Twelve adult female Göttingen mini pigs (Ellegaard, Denmark), being 14–16 months of age and weighting around 20–29 kg, were used. The animals were kept in standard cages and fed on a special soft diet for mini pigs. The complete surgical procedure was performed under general anaesthesia (10 ml of ketamine hydrochloride (Ketalar® 50 mg/ml, Pfizer), mixed with 3 ml of midazolam (Dormicum® 5 mg/ml, Roche).

An additional local anesthetic was given (Xylocain Dental adrenalin 20 mg/ml + 12.5 mg/ml, Astra AB, Södertälje, Sweden) to reduce the dosage of the systemic anesthetic as well as to reduce the bleeding during surgery and alleviate pain after surgery. Within the first days after surgery (healing phase), the animals were monitored routinely and further analgesia was given if necessary. The whole study was accompanied and monitored by a veterinarian.

Implants

Test implants were made of titanium-zirconia (TiZr1317) and control implants of titanium (Ti, commercially pure, Grade 4) (Institute Straumann AG, Basel, Switzerland). All implants had a hydrophilic sandblasted and acid-etched implant surface (SLActive®, Institute Straumann AG, Basel, Switzerland) (Figure 1, A–D). Implants used for biomechanical (RT) evaluation had a diameter of 4.8 mm and a length of 6 mm (Standard Plus body, Institute Straumann AG, Basel, Switzerland) and provided with a squared head were (Figure 2A). A specially designed bone chamber implant (ø 4.2 mm, L 5.0mm) was used for histologic evaluation (Figure 2B).

Surgery and Experimental Protocol

The experimental model used in the present study has been described in detail elsewhere.[12]The mandibular premolars (P1, P2, P3) and the first molar (M1) were extracted prior to the study. After 3 months of healing, a total of 36 RT implants and 36 bone chamber implants were implanted in the 12 mini pigs. Each hemimandible received three randomly allocated RT implants on one side (Figure 3A) and three bone chamber implants on the contralateral side (Figure 3B) in a split-mouth design, according to the scheme (Ti-TiZr-Ti and TiZr-Ti-TiZr).

The RT implants were placed such that the squared head was located above the bone level. Protective caps were applied to prevent bone from growing around the shaft. The bone chamber implants were placed such that the coronal margin of the implant was even at the bone level. The flaps were closed with absorbable sutures.

Termination of the Experiment

At the end of the 4-week healing period, the animals were sacrificed after general anesthesia with an

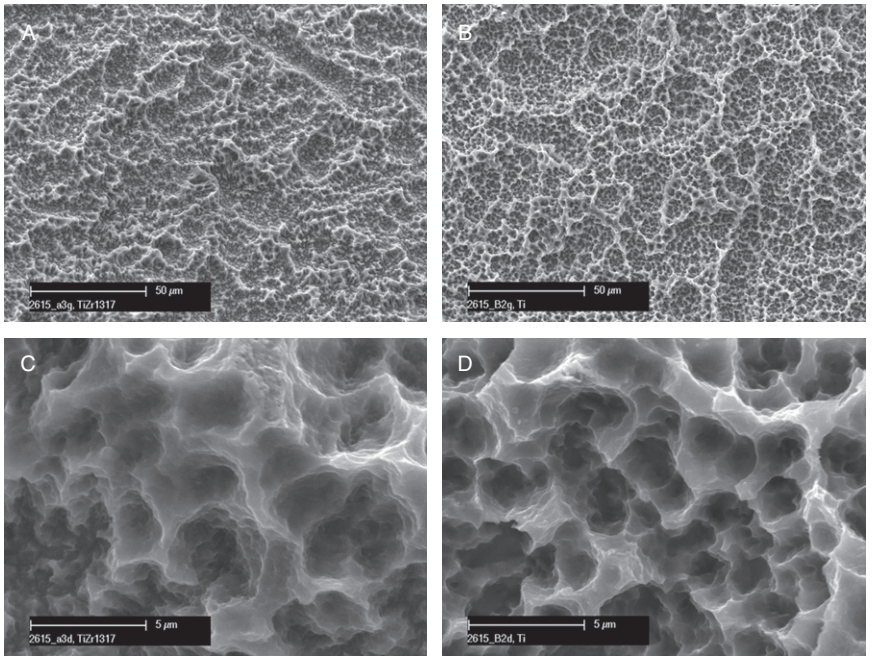

Figure 1 Scanning-electron microscopy images of the two surfaces used in the study. Overview of (A) TiZr1317 and (B) Ti surfaces. Bar = 50 um. Detail of (C) TiZr1317 and (D) Ti surfaces. Bar = 5 um.

intracardiac injection of a lethal dose of sodium pentobarbital. Immediately after sacrifice, the mandibles were excised and the left and right hemi-mandibles were separated with a bandsaw. The hemi-mandible with bone chamber implants was immersed in 4% formaldehyde solution for histologic processing. The other hemi-mandible was used for biomechanical evaluation.

RT Evaluation

Immediately after sacrifice, the soft tissues in the edentulous areas of the hemi-mandible and the protecting caps were removed to expose the head of the integrated implants. RT testing was accomplished by performing a counterclockwise rotation at a rate of 0.1°/sec. using a biaxial hydraulic testing machine (Instron, Bucks, UK) as described elsewhere.[10] The resulting torque–rotation curve was analyzed to determine the RT. The RT was defined as the maximum torque measured. In the case of a nonexistent failure point, the intersection between the curve and a line with a parallel offset of 0.72 degrees from the linear portion of the torque–rotation curve was selected. Interfacial stiffness was defined as the slope

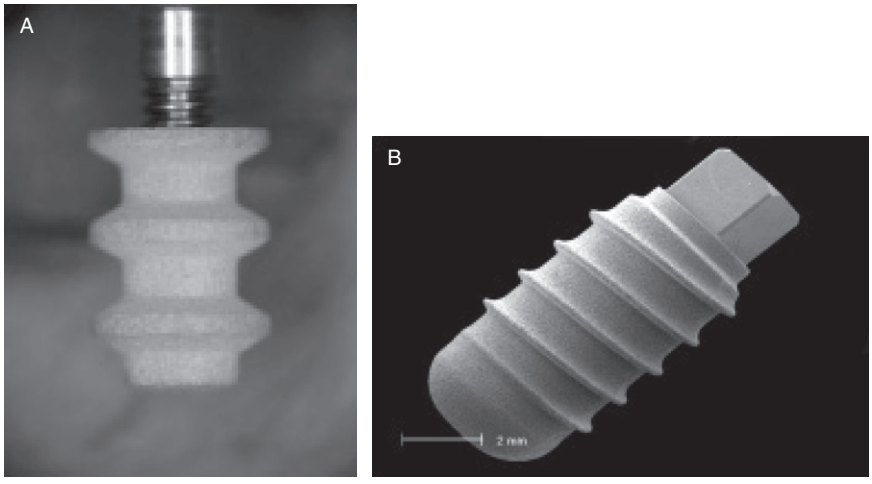

Figure 2 Showing the designs of experimental implants used for (A) removal torque and (B) histology.

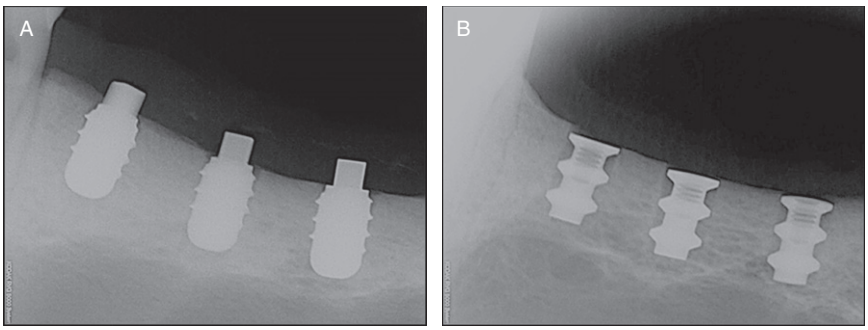

Figure 3 Postoperative radiographs of the pig mandible showing three implants used for (A) removal torque in one side and (B) histology in the other side.

(N-cm/°) of the linear part of the torque–rotation curve; this was calculated using linear regression analysis over the linear portion of the curve.

Histology

In the laboratory, the mandibles were divided in smaller sections comprising one implant and surrounding bone tissue and immersed in new buffered 4% formaldehyde solution. After 2 weeks, the specimens were dehydrated in a graded series of ethanol and embedded in light curing resing (Technovit 7200 VCL, Kulzer, Friedrichsdorf, Germany). Sections were taken through the longitudinal axis of each implant by sawing and grinding in mesio-distal direction (Exakt Apparatebau, Norderstedt, Germany). The sections, about 10 µm thick, were stained with toluidine blue and 1% pyronin-G. A qualitative analysis was performed with a light microscope (Nikon Eclipse 90i, Teknooptik AB, Huddinge, Sweden), evaluating the following criteria: native bone, new bone, and soft tissue.

Histomorphometric Measurements

Computer-assisted histometric measurements were obtained using an automated image analysis system (VisioMorph – Visiopharm Integrator System®, Visiopharm, Hørsholm, Denmark), coupled with a video camera (Nikon Digital Sight DS-5Mc) mounted on a light microscope (Nikon Eclipse 90i). Digital pictures of all 144 chambers (36 implants × 4) were taken with ×4 magnification and named with C1 and C2 for the coronal located chambers and A1 and A2 for the apical located chambers (1: right, 2: left) (Figure 4A).

The limits of all chambers were manually marked and the area within defined as region of interest (ROI, Figure 4B). On one picture, the ROI was manually segmented by marking implant areas (black = marked yellow), bone area (blue = marked red), and empty area (white = marked light blue) (Figure 4C). An automatic classification based on the manual segmentation was applied on all pictures by batch processing. Subsequently, all 144 pictures were checked one by one for successful segmentation and corrected when necessary.

The total number of pixels allocated to bone and to empty within the ROI area was counted by the software to calculate the bone area ratio The "Interface length" – calculation was chosen to measure the length of the implant-bone and the implant-empty interface. Based

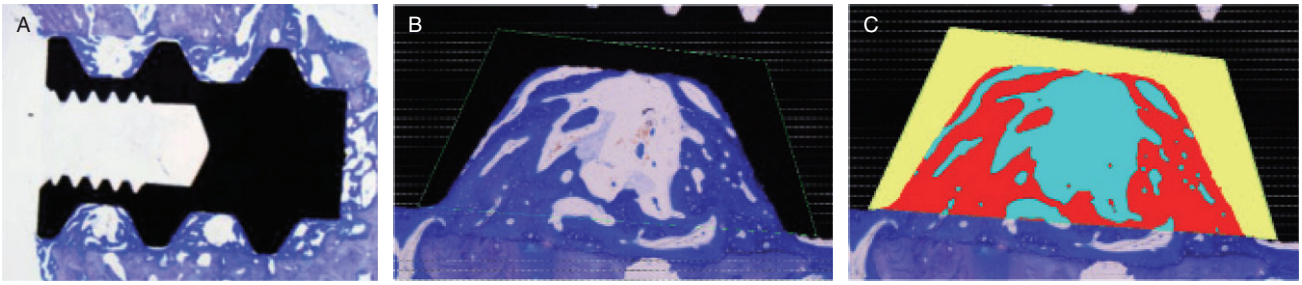

Figure 4 Showing the automated image analysis technique. (A) Digitized light micrograph of a bone chamber specimen. Note that each implant consists of four chambers (frame). (B) The limits of the chamber were marked manually (dotted frame). (C) The different components were segmented into different colors (ie, *yellow* = implant; *red* = bone; *cyan blue* = soft tissue). The image analysis system calculated the total area of the chamber and the areas of bone and soft tissue as well as bone contact to the implant.

on these values, the bone/implant interface ratio was calculated. All measurements were done blinded.

Statistical Analyses

Data analysis was performed with SAS software (SAS Institute, Cary, NC, USA). Measured parameters were summarized in terms of mean values and standard deviations. A paired *t*-test and the signed rank test were performed to look at the one to one relationship between the biomechanical outcomes (max. RT and stiffness) and the type of material (Ti or TiZr). The measurements of the two implants with the same material in the same animal were first averaged and then compared with the single measurement of the other material. Similarly, the measurements for all four chambers and for the two implants of the same material in the same animal were first averaged and then compared with the measurement of the single implant of the other material when evaluating the histomorphometric outcomes. Mixed model regressions were carried out to determine the tolerance level for equivalence between the test and control implants when the means were adjusted for animal, implant position and chamber effects. The calculated effect sizes of implantation levels and implant type and other factors resulting from the above regressions were adjusted for multiple comparisons using Dunnett's correction.

RESULTS

Clinical Observations

The experimental period was uneventful as none of the 12 animals showed signs of general health impairments during surgery, clinical follow-up, or at sacrifice.

RT Evaluation

The TiZr1317 implants revealed a significantly higher mean value for peak RT (230.9 ± 22.4 Ncm) than Ti implants (204.7 ± 24.0 Ncm) ($p = 0.013$) (Figure 5). Similarly, the mean stiffness (48.5 Ncm/°) of TiZr1317 implants was statistically significantly higher than that of the Ti implants (41.7 Ncm/°)($p = 0.029$). When comparing the implants within each animal the TiZr1317 implants always showed higher RT values compared with the Ti implants with the exception of one outliner (Figure 6). No significant dependence related to side or position appeared.

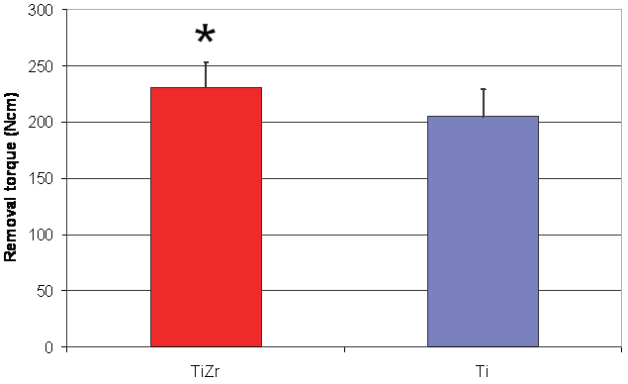

Figure 5 Showing the results from the removal torque test. $^*p < 0.05$.

Histology

No apparent dissimilarities could be discerned between the TiZr1317 and Ti implants. A clear difference was seen between the native bone of the implant bed and the new bone formed after surgery (Figure 7A–D). New bone apposition was observed at the cut surfaces of the native bone facing the implant bed. The native bone consisted of dense lamellar layers organized as typical haversian systems. The new bone was of woven type although noticeable areas of woven bone transforming to lamellar bone could also be seen. Newly formed bone trabeculae, originating from the edges of the experimental defect, were seen to follow the contour of the chamber toward its most centripetal aspect. Lining osteoblasts were recognizable on the outer surface of some bony trabeculae and along the implant surface. Early stages of haversian systems formation were also noticed driven by the presence of mature vessels.

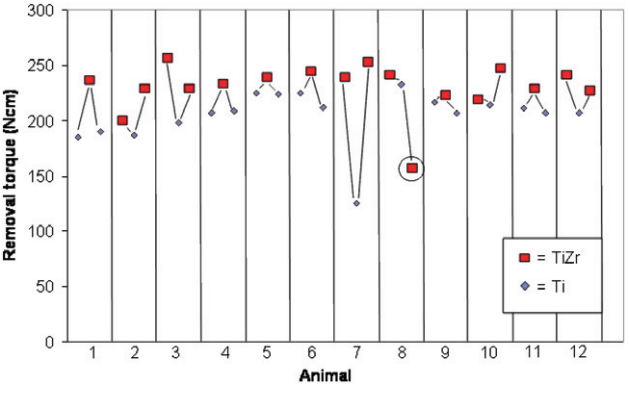

Figure 6 Showing the removal torque for each animal and implant. *Red* = test implants; *blue* = control implants.

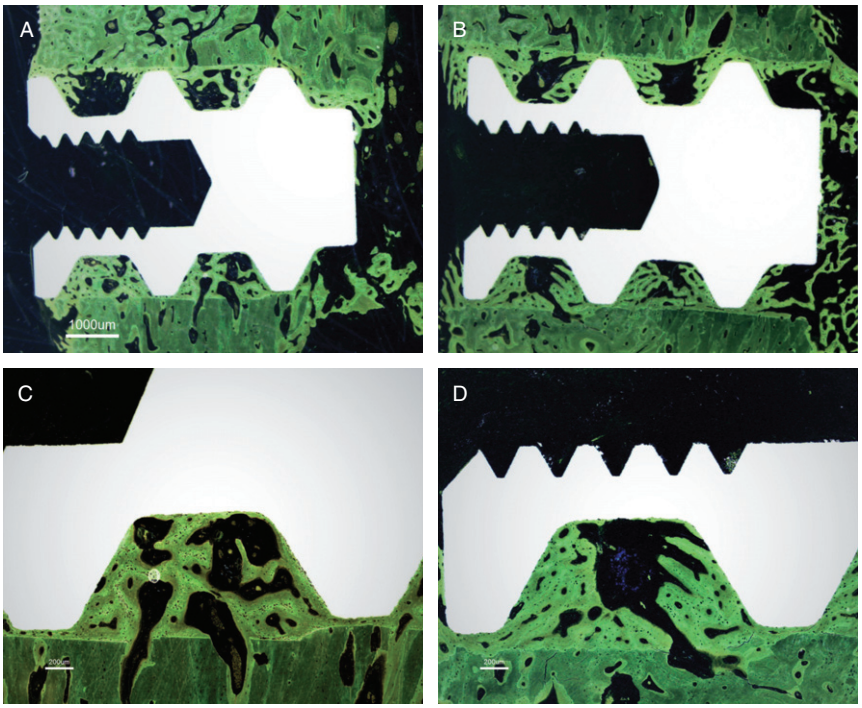

Figure 7 Light micrographs in inverted colors of bone chamber implants. Overviews of (A) a TiZr1317 specimen and (B) a Ti specimen. Note the clear differences between old (dark green) and new bone (light green). Bar = 1,000 um. (C) Detail showing one of the lower chambers. New bone formation (light green) is seen on the surfaces of the osteotomy into the chamber and along the implant surface. The formation of osteons can be seen (arrows). Bar = 200 um. (D) Detail of *B* showing one of the upper chambers. Similar bone formation as seen in *C* can be observed. Bar = 200 um.

Histomorphometry

A total of 36 implants with four chambers each were investigated. Two of the 144 chambers were excluded from the analysis because of the presence of native bone within the chamber.

Histomorphometric measurements revealed statistically significant more bone area within the chambers of the TiZr1317 implants (45.5 ± 13.2%) than within the chambers of the Ti implants (40.2 ± 15.2%) ($p = 0.023$) (Figure 8).

No difference was observed for the bone to implant contact measurements ($p = 0.96$) with 70.2 ± 17.3% for TiZr1317 implants and 72.3 ± 20.5% for Ti implants (Figure 8). The mixed model regression showed equivalence of test and control implants when the means were adjusted for animal, implant position and chamber effect ($p = 0.94$; difference between adjusted means 0.22; accepted tolerance −4.9).

DISCUSSION

The aim of this study was to test the hypothesis that TiZr1317 and Ti implants with identical hydrophilic sandblasted and acid-etched surfaces show comparable osseointegration and stability after 4 weeks healing in the mandible of mini pigs. The results from the study support the hypothesis because the novel TiZr1317 implants showed similar degrees of bone implant contact as the currently used Ti implant. Moreover, more bone area was found inside the bone chambers of the TiZr1317 implants, which also showed a significantly higher resistance to reverse torque than did the Ti implants. The latter findings indicated an even stronger

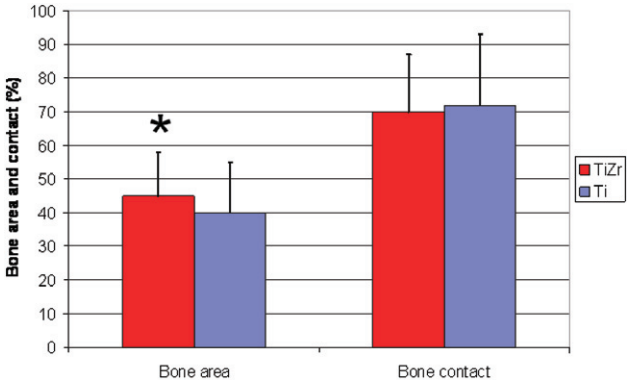

Figure 8 Results from morphometric measurements of bone area and bone contact. *$p = 0.023$.

bone tissue response to the novel as compared with the control implants.

The chemical and physical properties of the implant surface has a major influence on the structure of the implant–tissue interface and thus may influence the clinical performance of the implant. Titanium and zirconium are transition metals in the same group of the periodic table and have similar chemical properties. They exist both in two different crystal forms: the hexagonal close packed α phase and the body centered cubic β phase. In both cases, the α phase is the stable phase at room temperature and a phase transformation to the β phase takes place at elevated temperatures (883°C for Ti and 867°C for Zr). Surfaces with the characteristic structure as acquired by sandblasting and acid-etching cannot be obtained on many Ti-alloys like Ti_6Al_7Nb and Ti_6Al_4V because of the biphasic appearance of the alloys. The etching process leads to a selective enrichment of the β phase in case of Ti_6Al_7Nb and Ti_6Al_4V[13] and thus, no micro structure comparable with the SLA micro structure can be obtained. In addition, the enrichment of the β phase leads to Nb- and V-rich surfaces on Ti_6Al_7Nb and Ti_6Al_4V, respectively. However, TiZr1317 is an alloy with a monophasic α structure where sandblasting and acid-etching results in a topographically identical surface as on pure titanium implants.[2] Because of its superior mechanical properties, TiZr1317 have been identified as a material for production of thin implants and implant components subjected to high constrains, provided that the material shows a similar good biocompatibility as pure titanium. In a comparative in vitro investigation, Bernhard and colleagues[2] did not find any significant differences in the osteoblastic cell numbers cultured on Ti or TiZr1317 materials. Furthermore, they reported no influence of sandblasting and acid-etching on osteoblastic differentiation. The present animal study confirmed the high biocompatibility of the TiZr1317 implants when provided a hydrophilic sandblasted and acid-etched surface. If the whole framework of the present study is considered, TiZr1317 implants showed a stronger response to two out of three osseointegration parameters analyzed, ie, RT and bone area; whereas, the bone to implant contact was similar to that of the Ti implants after 4 weeks in the mandible of mini pigs. The reason for the differences can only be speculated upon. It could be hypothesized that the different performance is caused by slight differences in the topography or the surface chemistry. In fact, Zr-containing alloys show even better corrosion resistance than pure Ti.[14] Further studies are needed to explore the properties of the bone generated at the surface and on the surrounding environment of dental implants prepared from TiZr alloy with a hydrophilic sandblasted and acid-etched surface.

All implants used in the present study were packaged like the commercial implants with the same surface, ie, stored in an isotonic saline solution. All implants showed hydrophilic properties and attracted blood during implant insertion. No difference was noted in the clinical behavior (insertion, primary stability) between test and control implants at the time of surgery. Implants with the same body as the commercial implant of (Standard Plus, Institute Straumann AG, Basel, Switzerland), but with a squared head were used for biomechanical evaluation. The TiZr1317 implants showed significantly higher RT values than the Ti controls (mean value 230.9 Ncm and 204.7 Ncm, respectively). These values are numerically higher than those of Ferguson and colleagues[10] using the same Ti implant but placed in the maxilla of mini pigs. They found a mean RT value of 171 Ncm after 4 weeks, which most likely reflected the lower density of the maxillary bone.

A specially designed bone chamber implant, as is also used by other authors, was used for histologic evaluation.[15] The implant is stabilized by press-fit into the osteotomy in order to create a secluded and well-defined space for the study of new bone formation. In the present study, two chambers were excluded from the analysis because of the presence of native bone inside the chamber. After 4 weeks of healing, the chambers were filled with newly formed bone to varying extents which corroborate with the findings of previous studies using this technique. The new bone originated from the cut surfaces of the native bone and was also seen to follow the contour of the implant surface. The presence of lining osteoblasts on the surface and on the thin rims of newly formed bone, indicated so-called contact osteogenesis.[4,16] This concurs with the findings reported by Buser and colleagues[9] when evaluating the same Ti surface in the maxilla of mini pigs by using an implant identical to the control implant in the present study. Other studies using other modified surfaces have described the same phenomenon.[4–7]

CONCLUSIONS

It is concluded that the TiZr1317 implant with a hydrophilic sandblasted and acid-etched surface showed similar or even stronger bone tissue responses than the Ti control implant.

ACKNOWLEDGMENT

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