Bone Tissue Responses to Surface-Modified Zirconia Implants: A Histomorphometric and Removal Torque Study in the Rabbit

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ABSTRACT

Background: Zirconia ceramics are biocompatible and have mechanical properties that make them suitable as materials for dental implants. Little is known about how surface modification influences the stability and bone tissue response to zirconia implants.

Purpose: The objective of the investigation was to histologically and biomechanically evaluate the bone tissue response to zirconia implants with two different surface modifications in comparison with machined, nonmodified zirconia implants and oxidized titanium implants.

Materials and Methods: Threaded zirconia implants with a diameter of 3.75 mm with either a machined surface (Zr-Ctr) or one of two surface modifications (Zr-A and Zr-B) were manufactured. Oxidized titanium (Ti-Ox) implants 3.75 mm in diameter were also used. The implants were characterized with regard to surface topography using an interferometer. Twelve rabbits received 96 implants using a rotational scheme, two in each tibia and two in each femur. The implants in six rabbits were subjected to removal torque (RTQ) tests after a healing period of 6 weeks. The implants in the remaining six animals were removed en bloc for light microscopic analysis. Back-scatter scanning electron microscopic (BS-SEM) analyses were used to evaluate the state of the bone-implant interface at the modified zirconia implants after RTQ testing.

Results: The Ti-Ox and Zr-A implants showed the highest surface roughness, followed by the Zr-B implants and, finally, the Zr-Ctr implants. The nonmodified ZrO₂ implants showed statistically significant lower RTQs than all other implants. No significant differences in bone-implant contact or bone area filling the threads were observed. BS-SEM showed intact surface layers of the surface-modified implants after RTQ testing and revealed fracture of the interface bone rather than a separation.

Conclusion: The present study showed a strong bone tissue response to surface-modified zirconia implants after 6 weeks of healing in rabbit bone. The modified zirconia implants showed a resistance to torque forces similar to that of oxidized implants and a four- to fivefold increase compared with machined zirconia implants. The findings suggest that surface-modified zirconia implants can reach firm stability in bone.

KEY WORDS: dental implants, histology, rabbit, removal torque, zirconia
No mechanical problems, such as fractures of the implant material, were reported. In addition, histology showed high degrees of bone-implant contacts. Their findings are in line with those of Kohal and colleagues, who compared custom-made titanium and zirconia implants supporting single crowns in the maxilla of six monkeys. One case report from the same group demonstrated the clinical use of a custom-made zirconia dental implant in the anterior maxilla. However, no follow-up data were presented. Thus, the available documentation indicates that zirconia ceramics are suitable materials to be used as dental implants. Turning of zirconia rods results in a relatively smooth surface, and modern implant research shows that a rough surface topography is desirable to enhance the bone integration process.

The aim of the present animal investigation was to study the bone tissue response to zirconia implants with two different porous topographies.

**MATERIALS AND METHODS**

**Animals and Anesthesia**

Twelve female New Zealand White rabbits (> 8 months old) weighing between 3.5 and 4 kg were used in the study. The animals were kept in a purpose-designed room and were fed ad libitum with a standard diet. The study was approved by the Animal Research Ethics Committee at Göteborg University, Sweden.

The animals were anesthetized by intraperitoneal injections of diazepam (Kabi Pharmacia, Helsingborg, Sweden) at a dose of 1.5 mg/kg body weight and intramuscular injections of fluanisone and fentanyl (Hypnorm Vet, Janssen, Sanderton, England) at a dose of 0.2 mg/kg body weight. In addition, approximately 2 mL of 2% lidocaine with epinephrine 12.5 µg/mL (Xylocaine with adrenaline, AstraZeneca, Södertälje, Sweden) was used as local anesthesia.

**Implants and Surface Characterization**

Seventy-two threaded zirconia ceramic implants with three different surface topographies were used in the study (24 of each surface) (Figure 1). In brief, cold zirconia powder (TZ-3YSB-E, Tosoh Corporation, Tokyo, Japan) was isostatically pressed to rods. The rods were presintered and then turned into threaded implants. The implants had a 6 mm–long threaded part, 3.75 mm in diameter, and a 3 mm–high squared head for insertion and removal torque (RTQ) tests (see Figure 1). To achieve a porous surface, the implants were coated with slurry containing zirconia powder and a pore-former (patent application SE0302539-2). For the study, two slurries with different pore-formers were used, which gave different surface structures, denoted Zr-A and Zr-B. After the coating was applied, the implants were sintered to full density, under which the pore-former burned off and left a porous surface. Noncoated implants treated in the same way as the coated implants, with the exception of the coating process, were used as controls (Zr-Ctr). In addition, 24 modified oxidized titanium (Ti-Ox) implants (TiUnite™, Nobel Biocare AB, Göteborg, Sweden) were also used (see Figure 1). These implants had been modified by removing the cutting edges and bone chambers at the implant apex. The threaded part was 6 mm, the same as for the ceramic implants. This implant did not have a squared head but was placed and removed by using a Stargrip™ device (Nobel Biocare AB).

One implant of each type was photographed in a scanning electron microscope (Figure 2) and examined in an interferometer (MicroXam, Phase-Shift, Tucson, AZ, USA). Measurements were made in three threads at three locations: thread bottom, midflank, and on the tip of the thread. Thus, each parameter was described as the mean of nine measurements with standard deviations. The following parameters were used to characterize the surface topography: (1) Sa value, the absolute values of the surface departures from a mean plane; (2) Sds value, the number of peaks per area unit; and (3) Sdr value, the ratio between the developed surface area and a flat reference area. In brief, the Ti-Ox and Zr-A implants showed the highest surface roughness, followed by the Zr-B implants and, finally, the Zr-Ctr implants (Table 1).
Figure 2  Scanning electron micrographs of the different surfaces used in the study. A, Zr-Ctr; bar = 50 µm. B, Zr-Ctr; bar = 10 µm. C, Zr-A; bar = 50 µm. D, Zr-A; bar = 10 µm. E, Zr-B; bar = 50 µm. F, Zr-B; bar = 10 µm. G, Ti-Ox; bar = 50 µm. H, Ti-Ox; bar = 10 µm.
Surgery

All surgery was performed under aseptic conditions. The distal femoral condyles and proximal tibial metaphyses on both sides were used as experimental sites. The bone was exposed via incisions through skin and fascia. Implant sites were prepared using Brånemark System® drills (Nobel Biocare AB). In brief, 1.8, 2, and 3 mm drills were used and the implants were placed after threading with a screw tap. No countersink preparation was performed. Each rabbit received eight implants (in total 96 implants), two in each tibia and two in each femur according to a predefined rotating scheme. The ceramic implants were placed with one thread visible above the cortex and the titanium implant with the head on top of the cortex. Fasciae and skin were sutured in separate layers with resorbable sutures.

Specimen Preparation and Analysis

All animals were sacrificed after a healing period of 6 weeks by intravenous injections of pentobarbital (Pentobarbitalum, Apoteksbolaget, Uppsala, Sweden).

Six animals were subjected to RTQ tests using a specially designed electronic device. The instrument involved an electric motor with a strain gauge mounted on a metal frame. The instrument was connected to the squared head of the ceramic implants or a Stargrip for the titanium implants. A fixed rotation rate was applied until failure of the bone-implant interface occurred. The peak RTQ was registered and used for statistical analyses.

The remaining six animals were used for histology. The implants with surrounding bone tissue were removed en bloc and fixated by immersion in 4% buffered formaldehyde. The specimens were then dehydrated in a graded series of ethanol and embedded in light-curing resin (Technovit 7200 VLC, Heraeus Kulzer GmbH & Co., Wehrheim, Germany). One central section of each implant was taken using a sawing and grinding technique (EXAKT Apparatebau GmbH & Co., Norderstedt, Germany). Each section was ground to a thickness of about 10 µm and stained with 1% toluidine blue and 1% pyronin G.

The sections were viewed and analyzed in a light microscope (Nikon Eclipse 80i, Tekno Optik AB, Göteborg, Sweden) connected to a personal computer with software for morphometry (Easy Image Analysis 2000, Tekno Optik AB, Göteborg, Sweden). The degree of bone-implant contact and the bone area occupying the threads were measured. Mean values based on measurements of all threads for each implant were used to calculate mean values for each implant type and parameter.

One specimen of each implant type that had been subjected to RTQ tests was fixated, dehydrated, and embedded in light-curing resin. The blocs were divided, polished, and coated with a thin gold layer. The specimens were examined in a JEOL JSM-820 scanning electron microscope (JEOL AB, Sollentuna, Sweden) in a back-scatter mode with regard to the state of the bone-implant interface.

Statistical Analyses

The data obtained were analyzed using a Mann-Whitney U test and were expressed as mean ± SD, and \( p < .05 \) was set for significance.

RESULTS

Clinical Observations

All implants achieved good primary stability. One ceramic implant showed a partial fracture of the squared head during insertion. The healing period was uneventful, and the experimental sites healed well during the 6 weeks.

RTQ Tests

The Zr-Ctr showed significantly lower RTQ values compared with all other implant types in both the tibia and the femur (\( p < .05 \)) (Figure 3). Although not statistically significant, the Zr-A and Zr-B implants showed higher RTQ values than the Ti-Ox implants. The RTQ values were higher in the femur than in the tibia for all implant types.

Histology

Gross examination of the light microscopic sections showed bone formation and integration of all implant types (Figures 4 to 7). The integration process was
mainly due to ingrowth of bone from the surroundings. The surface-modified zirconia and titanium implants also showed bone formation directly on the implant surface (see Figures 5C, 6C, and 7C). The surface layers of the Zr-A and Zr-B implants were easily distinguished (see Figures 5 and 6). It was evident that stainable biologic material occupied the porosities of the surface (see Figures 5C and 6C). Islets of mineralized bone were often seen in intimate contact with the surface layer. Active osteoblasts could be distinguished on the surface of this bone, giving an impression of bone formation at the implant surface and in a direction toward the surrounding tissues. A similar morphology was seen for Ti-Ox implants (see Figure 7C).

The morphometric measurements showed more bone contacts for Zr-A implants compared with Zr-Ctr implants in the three best threads in the tibia (Figure 8). No other statistically significant differences were detected for any of the measured parameters (see Figures 8 and 9).

Scanning Electron Microscopy

Scanning electron microscopy (SEM) of RTQ-tested implants showed intact surface layers of all implant types. Fracture of the zirconia ceramic could be observed at one location on each of the Zr-A and Zr-B implants. However, it was not clear if this was due to the RTQ testing or the preparation process. Bone tissue was still in contact with the Zr-A, Zr-B, and Ti-Ox surfaces, indicating fracture of the bone rather than a clean separation of the interface.

DISCUSSION

The present animal study showed a stronger bone tissue response and high resistance to RTQ for surface-modified zirconia implants after 6 weeks of healing in the rabbit. The histologic and biomechanical performance was the same as for oxidized titanium implants, which were used as controls. Zirconia implants with no surface modification were statistically less stable than the other implants. Although there was a tendency toward less bone contact with machined zirconia, there were no statistically significant differences, indicating a simi-

Figure 3 Results from the removal torque tests presented for femoral and tibial sites and as pooled. *p < .05 in comparison with Zr-Ctr implants. There were no other statistically significant differences.

**Figure 4** Light micrographs showing Zr-Ctr implants after 6 weeks of healing. A, Overview showing bone apposition toward the implant surface. Bar = 500 µm. B, Separation of bone tissue and the zirconia surface by a loose connective tissue. Bar = 200 µm. C, Detail showing solitary bone formation near the implant surface. The bone is separated from the surface by cells. Bar = 50 µm (stained with toluidine blue and pyronin-G).
lar good biocompatibility. This underlines the importance of a surface structure to achieve firm connection with the bone.

Osseointegration of threaded zirconia implants has been demonstrated in various animal models.2–5 Akagawa and colleagues compared the bone tissue response to loaded and unloaded zirconia implants in the dog mandible.3 Threaded implants were machined from zirconia rods and barrel polished. The authors reported high degrees of bone-implant contact 3 months after installation, with no differences between the groups. In a monkey model, the group evaluated the tissue responses to similar zirconia implants under different prosthetic conditions.4 The implants were inserted in the mandible and allowed to heal for 3 months. Thereafter, implants were loaded as single units, splinted with a bridge, or connected with natural teeth. Histologic evaluations 24 months after installation revealed osseointegration with no differences in bone contact or bone area ratios between the groups. However, one free-standing implant failed, and this group showed statistically fewer bone contacts after 12 months of healing. No mechanical problems, such as fracture of the implants, were reported, indicating the favorable mechanical

Figure 5 Light micrographs showing Zr-A implants after 6 weeks of healing. A, Overview showing contact between bone and the surface layer. Bar = 500 μm. B, Close-up showing an apparently intimate contact between bone and the surface layer. Bar = 200 μm. C, Bone is seen in intimate contact with the surface layer. Stainable biologic material extends from the bone into the porous layer. Bone is formed in a distal direction from the bone spiculae. Bar = 50 μm (stained with toluidine blue and pyronin-G).

Figure 6 Light micrographs showing Zr-B implants after 6 weeks of healing. A, Overview. Bar = 500 μm. B, Showing bone formation in contact with one thread. Bar = 200 μm. C, High magnification showing bone formation directly at the surface of the implant. Osteoid and a partly entrapped osteoblast/osteocyte indicative of active bone formation are seen on top of the bone island. Bar = 50 μm (stained with toluidine blue and pyronin-G).
properties of zirconia, which is in line with the experiences of Kohal and colleagues. These authors compared custom-made titanium and zirconia implants used to support metal crowns in the maxilla of six monkeys. Both implant types were sandblasted, and the titanium was also acid-etched. However, the surface topography was not measured or described. The implants were allowed to heal for 6 months prior to abutment connection. Metal crowns were cemented after 3 months, and the animals were followed for another 5 months. All implants achieved and maintained stability, and no mechanical problems were reported. Histology revealed no differences in the bone tissue response to the titanium and zirconia implants. In the present study, the SEM examinations revealed no fractures of the zirconia itself or the surface layers of the modified implants after RTQ testing in spite of the high RTQ measured.

It is well known that surface modification can enhance bone integration of titanium implants, which, in animal studies, can be observed as higher bone-contact ratios and greater resistance to RTQ than nonmodified implants. Apart from quantitative differences, there seem to be different integration mechanisms. Implants with “as-machined” surface have been described to be

Figure 7 Light micrographs showing Ti-Ox implants after 6 weeks of healing. A, Overview. Bar = 500 µm. B, Bone formation in one thread. Bar = 200 µm. C, Close-up showing similar solitary bone formation at the surface, as seen for Zr-A and Zr-B implants. Bar = 50 µm (stained with toluidine blue and pyronin-G).

Figure 8 Results from bone-implant contact measurements presented for femoral and tibial sites and for the three best threads of tibial implants. *p < .05 in comparison with Zr-Ctr implants. No other statistically significant differences were seen.

Figure 9 Results from bone area measurements presented for femoral and tibial sites and for the three best threads of tibial implants. No statistically significant differences were seen.
integrated by ingrowth from adjacent bone surfaces, whereas surface-modified implants also show bone formation directly on the surface. The mechanisms are not fully known, but the topography is likely of great importance, although the influence of surface chemistry should not be underestimated. The present implants, with a surface roughness similar to but a chemistry different from that of Ti-Ox implants, showed signs of contact osteogenesis. It was evident that osteoblasts formed bone in layers from the implant surfaces and toward the surrounding tissues. The light-transmitting properties of zirconia made it possible to evaluate the surface layer in high magnification and stainable biologic material could be distinguished deep into the surface layer of the modified implants. It is possible that this material was remnants from the initial fibrin clot. Retention of the initial blood clot at the implant surface is considered one important factor behind contact osteogenesis. A recent SEM evaluation of the bone–oxidized implant interface revealed intimate bone contact and ingrowth of bone into less than 2 µm wide pores.

Other ceramics, such as aluminum oxides, have been used as dental implants. Clinical follow-up studies on the Tübingen implant showed survival rates from 80 to over 90%. However, this implant was later withdrawn from the market, possibly owing to problems with mechanical failure. There is a bulk of evidence that zirconia ceramics are highly biocompatible and have the mechanical properties required to serve well as materials for dental implants. However, to the knowledge of the present authors, the clinical experience of zirconia implants is restricted to one case report showing replacement of a central incisor with a custom-made threaded zirconia implant. Clinical trials are obviously needed to evaluate the performance of zirconia implants under clinical conditions.

The present study showed a strong bone tissue response to surface-modified zirconia implants after 6 weeks of healing in rabbit bone. The modified zirconia implants showed a resistance to torque forces similar to that of oxidized implants and a four- to fivefold increase compared with machined zirconia implants. The findings suggest that surface-modified zirconia implants can reach firm stability in bone.

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