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Biomechanical and Histomorphometric Evaluation of Osseointegration of Fusion-Sputtered Zirconia Implants

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Keywords
Zirconia implants; fusion sputtering; biomechanical; removal torque; histometric.

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Abstract

Purpose: The aim of this study was to evaluate osseointegration of fusion-sputtered zirconia implants in comparison with sandblasted, acid-etched titanium implants in a biomechanical and histomorphometric study.

Materials and Methods: Sixty zirconia implants were manufactured using CAD/CAM. Half received fusion sputtering surface treatment through spraying the green body implants with a jet of zirconia suspension. Standard Ti implants of the same shape and dimensions served as control. Thirty adult New Zealand white male rabbits were used in this study. Each animal received one fusion-sputtered and one Ti implant in one femur site and control zirconia in the other, for a healing period of 4, 8, and 12 weeks. At each healing time, a removal torque (RTQ) test was used to assess implant stability, while histological and histometric analyses were used to evaluate osseointegration.

Results: Fusion-sputtered zirconia implants demonstrated a statistically higher mean RTQ than control zirconia. When compared to Ti, however, although still higher, the differences were not significant. Histomorphometric evaluation revealed significantly greater bone-implant contact for fusion-sputtered zirconia implants compared to Ti after 4 and 8 weeks of healing time; however, at 12 weeks, the difference did not reach statistical significance. There were no significant differences in the measured bone density between fusion-sputtered and Ti implants, although the difference was significant when compared to the control zirconia.

Conclusion: Fusion-sputtered zirconia implants demonstrated a degree of osseointegration and interfacial biomechanical stability comparable to Ti implants.

The search for an optimum dental implant material has long been a point of concern for scientific research. The most often used material is titanium, due to its well-documented biocompatibility,[1] high success rate, and various applications in the oral cavity.[2,3] The main drawback is its gray color, which may pose a problem in esthetically critical areas of the mouth, when paired with unfavorable soft tissue response or thin gingival biotype.[4-6]

Zirconia was introduced to implant dentistry as a potential metal-free framework material, mainly due to its tooth-like color and ability to transmit light, which make it esthetically attractive, coupled with its outstanding mechanical properties like high flexural strength and fracture toughness.[7,8] Another advantage of zirconia is the fact that it displays a significantly reduced plaque affinity, thus reducing the risk of inflammatory changes in the surrounding soft tissue.[9] Histological observations and various animal studies have shown that zirconia implants osseointegrate to the same extent as Ti implants, if not better.[10-13] As the success of any implant material is interpreted by its degree of osseointegration, zirconia surface modifications have been applied and investigated to enhance bone apposition and implant stability. Even though the optimal surface modification has not yet been found, various approaches have been used in an effort to improve surface properties of zirconia. These approaches include airborne-particle abrasion, acid etching with hydrochloric or hydrofluoric acids, plasma spraying,[8,13] aggregation of bioactive materials such as hydroxyapatite,[12] and more recently, UV radiation, which has been used to increase the hydrophilic properties of zirconia implants.[14]

In the same vein, the fusion-sputtering technique evolved as an innovative surface treatment used to transform the relatively smooth and dense surface of zirconia into a micro-rough

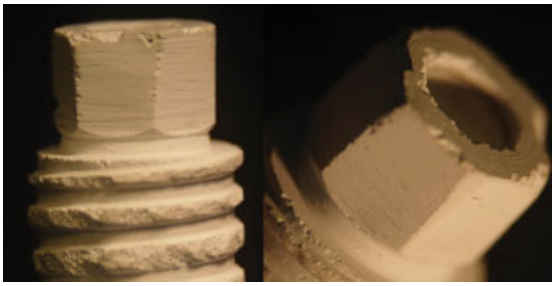

Figure 1 Zirconia implant design with external hex implant head.

retentive surface.[15,16] In this technique, a jet of zirconia suspension is sprayed under pressure over the surface of green body (un-sintered) zirconia implants. The fine zirconia particles become attached on the surface of the implant and eventually become fused to the surface after sintering.

The aim of this animal study was to investigate osseointegration of fusion-sputtered zirconia implants in comparison with sandblasted and acid-etched (SLA) Ti implants in a biomechanical and histomorphometric study. The proposed hypothesis was that fusion sputtering would improve the performance of zirconia implants compared to untreated surfaces.

Materials and methods

Fabrication of zirconia implants

Sixty threaded custom-made zirconia implants were fabricated by milling zirconia blocks prepared by compression molding of 50 μm zirconia powder (E grade 3 mol Y-TZP, Toso Inc, Tokyo, Japan). Modified CAD/CAM (Cercon, Degudent, Hanau Wolfgang, Germany) was used to mill the required shape of the implants. All implants had the same design with a standardized diameter of 3.7 mm, a length of 8 mm, and spiral threads with 0.9 mm pitch and 0.5 mm depth. An external hex configuration was incorporated into the implant head design to provide mechanical connection to the seating driver and to facilitate removal torque (RTQ) testing (Fig 1).

Fusion-sputtering technique

Half of the prepared zirconia implants received fusion-sputtering surface treatment through spraying a suspension of zirconia mixture composed of 5 g ultrafine zirconia powder (1–5 μm) and 10 ml ethyl alcohol (70%). To ensure good adherence, 1 ml of polyethyl alcohol was added to the mixture. The slurry was mixed over a stirring plate to produce a homogenous mixture that was sprayed under a pressure of 1 bar on the outer surface of the partially sintered zirconia implants. After sintering, the sprayed zirconia particles became fused to the outer surface of the implants. Surface topography of fusion-sputtered zirconia implants was qualitatively examined using scanning electron microscopy (SEM) (Jeol, JSM-5300, Tokyo, Japan). The measured surface roughness (Ra) of fusion-sputtered zirconia implants ranged between 10 and 14 μm (Marsurf PS1, Mahr GM6H, Gottingen, Germany). Commercially available sandblasted and acid-etched titanium implants with a 3.7 mm diameter and an 8 mm length (SLA, Tapered SP MTX, Zimmer Dental, Carlsbad, CA) were used as control.

Experimental procedures

The protocol of the study was approved by the Ethics Committee of Alexandria University, Egypt. Thirty adult New Zealand white male rabbits (6 months old) weighing approximately 4.0 to 5.0 kg were included in this study. The animals were housed in individual stainless-steel cages in an animal house. Temperature was maintained at 24 $\pm$ 4°C with a relative humidity of ~50 to 65%. Proper ventilation and a 12-hour light/dark cycle were applied. The animals had free access to water and a standard diet throughout the study. Animals were routinely observed and acclimatized to the environment of the animal facility for at least 1 week before surgery to ensure adequate health and stability.

Surgical procedures

All surgeries were performed under sterile conditions in a veterinary operating theater by one experienced surgeon. The animals were operated on under general anesthetic induced by intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). In the areas exposed to surgery, 1 ml of lidocaine infiltration anesthesia (Lidocaine 2%, 1:100,000 epinephrine) was injected. The hind limbs of the animal were shaved, washed, and disinfected with iodine before being isolated with surgical drapes. The rabbit femur condyles on both sides were surgically exposed via a skin incision, blunt dissection of the muscles, and elevation of the periosteum. The implant site was prepared using sequential water-cooled surgical drills with increasing diameter at 700 rpm (Zimmer surgical kit, Zimmer Dental). The rabbit femur on one side received one fusion-sputtered zirconia implant and one Ti implant, while the other side received a control zirconia implant, predetermined in a randomized scheme.

After implants were screwed, fascia and skin were sutured in separate layers with resorbable sutures (Vicryl Rapide 5; Ethicon Inc., Somerville, NY). Animals were then moved to recovery rooms and monitored for any possible complications until full recovery. Postoperatively the animals were inspected for signs of wound dehiscence or infection. A single dose of long-acting analgesic was administered (Buprenorphine hydrochloride, 0.04 mg/kg i.m), and a 7-day course of a broad spectrum antibiotic (Amoxylline 5 mg/kg i.m) was administered for infection control. Animals were sedated and euthanized by IV injections of sodium pentobarbital (100 mg/kg) after a healing period of 4, 8 and 12 weeks. Bone blocks containing the integrated implants were dissected from the animals, unnecessary fragments of bone and soft tissue were removed, and the specimens were prepared for subsequent investigations.

RTQ test

Five animals at each healing time were subjected to RTQ testing. Immediately after euthanization, bone segments containing the integrated implants were fixed on a bench mount, and the external hex at the implant head was securely connected to an electric driven handpiece (W&H WI 75E/ICM, Zimmer Dental) using the supplied connection. The implants were removed under reverse torque rotation, and the peak RTQ value was digitally registered for each implant (Aseptico surgical motor, AEU

6000, Aseptico Inc, Woodinville, WA). The test was performed after 4, 8, and 12 weeks of healing time.

Histological analysis

Bone specimens from the remaining animals at each evaluation time were immediately fixed in 4% buffered formaldehyde. Then, the specimens were dehydrated in graded ethanol solutions using a dehydration system under agitation and vacuum. Specimens were then defatted in xylene and embedded in transparent chemically polymerized methyl methacrylate resin (Methyl methacrylate 99%, Sigma-Aldrich, Steinheim, Germany). After polymerization, the specimens were cut along the long axis of the implants in a coronal-apical plane using a diamond-coated saw rotating in a micro-sectioning system (Micracut 150 precision cutter, Metkon, Bursa, Turkey) followed by grinding and polishing using 800 grit silicon carbide paper. At least three middle sections were obtained for each implant. The most central section from every implant was stained using Stevenel's Blue and Van Gieson's Stains. The section was imaged and analyzed using light microscopy (Olympus BX 61, Hamburg, Germany) equipped with a high-resolution camera (E330, Olympus, Imaging Corp, Beijing, China).

Histometric analysis

Histometric analysis was performed by one experienced examiner using an image analysis software system (Olympus Cell^{M} & Cell^{R}, version 3.3, Olympus Soft Imaging Solutions). High-resolution digital images were recorded for each specimen. Bone-implant contact (BIC) ratio was measured as the ratio of direct bone contact to the implant surface calculated as a percentage of the total implant perimeter. Bone density within and outside the implant threads was calculated using the software system's image separation feature under higher magnifications. The percentage of mature bone (stained red) was calculated against the percentage of immature bone (stained green) within and outside the implant threads. The area within the implant threads was defined by placing a borderline at the tips of the threads, parallel to the implant length (BD-bt), while the area outside was represented by a rectangular shape in the area immediately outside the threads (BD-ot).

Statistical analysis

Examiner reliability for the histometric analysis was cross checked by reevaluation of randomly selected digital images by another expert examiner. The recorded concordance correlation coefficient ranged from 0.85 to 0.90, indicating high reliability for all measured parameters. The data obtained were expressed as mean ± standard deviation and analyzed using one-way ANOVA and Bonferroni post hoc test for pairwise comparisons ($\alpha = 0.05$) (SPSS 15.0, SPSS, Chicago, IL).

Results

All animals survived the surgical procedures with an uneventful healing period and were available for evaluation. All implants appeared to be osseointegrated and were clinically stable upon retrieval with no signs of inflammation or mobility.

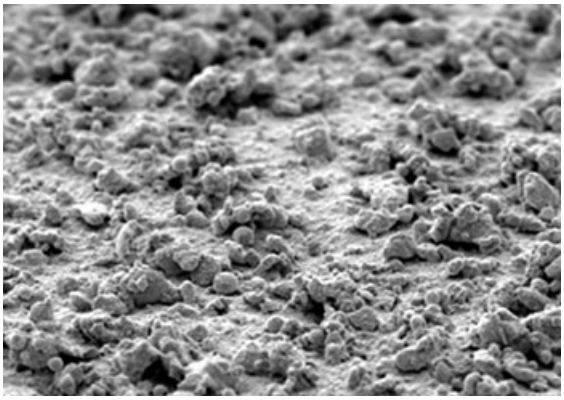

Figure 2 SEM micrograph of fusion-sputtered zirconia implant demonstrating granular surface composed of round zirconia particles fused to the outer surface of the implant (10,000×).

SEM

Scanning electron microscope images revealed a rough microstructure for the fusion-sputtered zirconia implant surface with a granular surface composed of round particles fused to the outer surface of the implants. The surface granules had an average height of 14 to 18 μm, which accounts for increased surface roughness measurements (Ra = 14 ± 5). Surface granules demonstrated even distribution and an identical morphological pattern along the entire implant surface (Fig 2).

RTQ

At all tested time intervals, fusion-sputtered zirconia implants demonstrated statistically higher mean RTQ values than that of control zirconia; however, when compared to titanium implants, although still numerically higher, the differences did not reach statistical significance. At 4 weeks, the mean RTQ for fusion-sputtered zirconia implants was 46.24 ± 2.24 N cm, while the mean RTQ for Ti implants and control zirconia were 43.58 ± 2.37 N cm and 35.72 ± 3.75 N cm, respectively. At 8 weeks, the recorded results were 78.08 ± 2.83 N cm, 74.26 ± 3.39 N cm, and 63.10 ± 2.33 N cm for fusion-sputtered zirconia, Ti, and control zirconia, respectively. At 12 weeks the mean RTQ revealed a slight increase for all tested implants (Table 1).

Table 1 Removal torque values (N cm) of tested groups (Mean ± SD)

Implant	RTQ 4 weeks[a]	RTQ 8 weeks[b]	RTQ 12 weeks[c]
Fusion-sputtered zirconia	46.24 ± 2.24A	78.08 ± 2.83A	78.70 ± 2.88A
Titanium	43.58 ± 2.37A	74.26 ± 3.39A	74.96 ± 3.72A
Control zirconia	35.72 ± 3.75	63.10 ± 2.33	63.64 ± 3.02

RTQ, removal torque.

Means followed by the same capital letters do not differ statistically.

[a]F (2, 12) = 18.13; $p < 0.001$. [b]F (2, 12) = 36.32; $p < 0.001$. [c]F (2, 12) = 29.46; $p < 0.001$.

Histological results

At 4 weeks of healing time, it was possible to observe the presence of newly formed bone trabeculae in direct contact with all implant surfaces. Active osteoblasts secreting osteoid matrix were also evident. No gaps, fibrous tissue, or foreign body reaction were observed at the bone/implant interface (Fig 3). Further increase in bone apposition on all studied implant surfaces could be observed after 8 weeks healing time (Fig 4). After 12 weeks, successful osseointegration of the zirconia as well as titanium implants was visualized with intimate contact of mature lamellar bone along the entire length of all implant surfaces (Fig 5). No interposition of an interfacial layer of soft tissue was detected.

Histometric results

For all implant types, the BIC values showed an increase from the 4th to the 12th week. Following a 4-week healing period, fusion-sputtered zirconia implants demonstrated significantly greater ($F = 24.9$, $P < 0.001$) BIC compared to both titanium and control zirconia implants. The mean BIC for fusion-sputtered zirconia implants was 69.66 ± 3.46

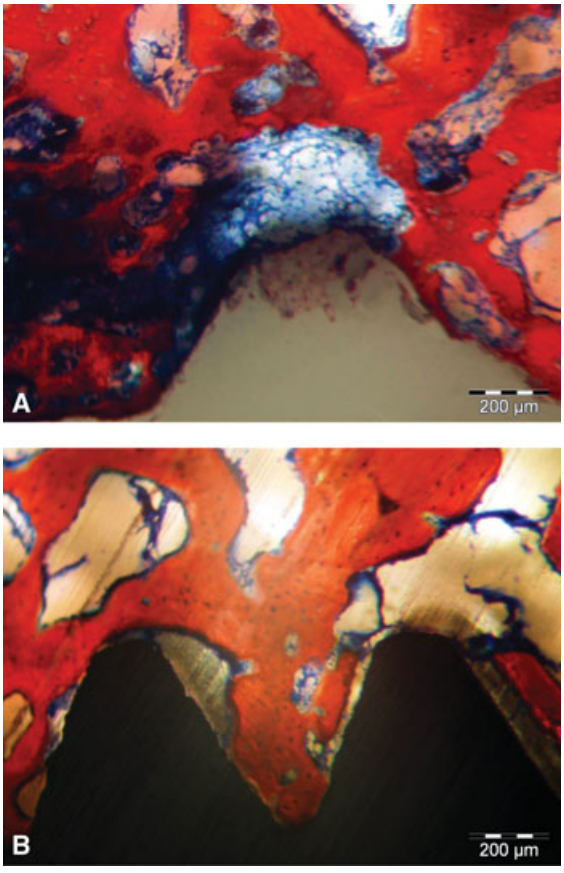

Figure 3 Micrograph of (A) fusion-sputtered zirconia, (B) Ti implants, after 4 weeks of implantation, showing newly formed bone in direct contact with implant surfaces (Stevenel's Blue and Van Gieson's Stain 220x).

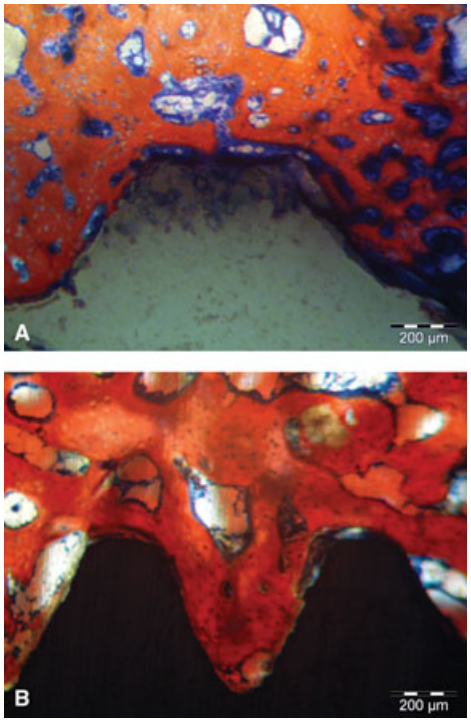

Figure 4 Micrograph of (A) fusion-sputtered zirconia, (B) Ti implants, after 8 weeks of implantation, showing increase in bone apposition on both implant surfaces (Stevenel's Blue and Van Gieson's Stain 220×).

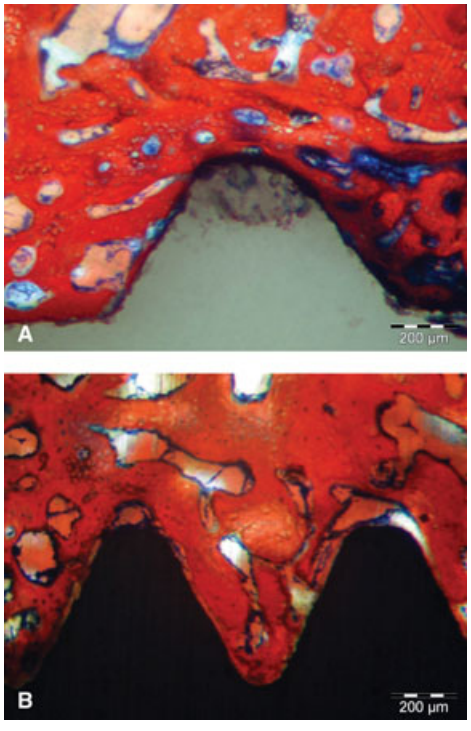

Figure 5 Micrograph of (A) fusion-sputtered zirconia, (B) Ti implants, after 12 weeks of implantation, showing complete osseointegration of implants with intimate contact of mature lamellar bone along the entire length of all implant surfaces (Stevenel's Blue and Van Gieson's Stain, 220×).

Table 2 BIC analysis of tested groups after 4, 8, and 12 weeks healing time (Mean ± SD)

Implant	BIC (%) 4 weeks[a]	BIC (%) 8 weeks[b]	BIC (%) 12 weeks[c]
Fusion-sputtered zirconia	69.66 ± 3.46	88.03 ± 2.94	89.09 ± 2.81A
Titanium	62.83 ± 1.97	82.94 ± 2.79	86.77 ± 3.09A
Control zirconia	56.94 ± 2.91	70.36 ± 2.88	74.76 ± 3.85

BIC: bone implant contact.

Means followed by the same capital letters do not differ statistically.

[a]$F(2, 12) = 24.9$; $p < 0.001$. [b]$F(2, 12) = 50.03$; $p < 0.001$. [c]$F(2, 12) = 27.46$; $p < 0.001$.

compared to 62.83 ± 1.97 and 56.94 ± 2.91 for Ti and control zirconia implants, respectively. After 8 weeks of healing time, the recorded results revealed the same pattern, as BIC for fusion-sputtered zirconia implants remained statistically higher ($F = 50.03$, $P < 0.001$) than that of Ti and control zirconia implants. At 12 weeks, both fusion-sputtered and Ti implants demonstrated comparable BIC values, which when compared, were not statistically significant (Table 2). There were no significant differences in the measured bone density within or outside the implant threads observed at the studied intervals between fusion-sputtered and Ti implants, although the difference was significant when compared to the control zirconia (Table 3).

Discussion

Superior mechanical properties, excellent biocompatibility, and tooth-like color make zirconia bioceramics suitable as a dental implant material.[7,8] Still, the influence of zirconia surface modification on osseointegration has not been extensively investigated. To improve surface properties of zirconia implants, two main approaches were used, either optimizing the microroughness using sandblasting and chemical etching, or by applying bioactive coatings such as hydroxyapatite and calcium phosphate. Implants with micro-scale surface roughness have demonstrated favorable results compared to implants with smooth and dense surfaces. Gahlert et al investigated zirconia implants with either a machined or a sandblasted surface and compared them with sandblasted and acid-etched titanium implant surfaces in the maxilla of minipigs. The machined ZrO_2 implants showed statistically significantly lower RTQ values than the other two implant types. The authors concluded that roughening the machined zirconia implants enhances their osseointegration.[8] Other animal and clinical studies also reported successful osseointegration of roughened zirconia implant surfaces comparable to Ti implants.[9,17-19]

A novel surface roughening procedure, fusion-sputtering technique, was investigated in this study. The technique is used to create a micro-rough surface through spraying the green body implants with a jet of zirconia suspension. This simple technique creates a micro-roughened surface and an increase in the total surface area of the implant without creation of any structural defects observed with airborne-particle abrasion and other techniques. The present study demonstrated a superior bone tissue response observed for fusion-sputtered zirconia implant surface as evident by higher values of BIC ratios and greater resistance to RTQ compared to as-sintered zirconia implants. The proposed hypothesis was thus accepted.

The micro-rough surface characteristics of fusion-sputtered zirconia implants enhanced bone apposition at the bone/implant interface and had a beneficial effect on the interfacial shear strength. Histological analysis of both Ti and zirconia implant surfaces revealed comparable osseointegration activity, suggesting that both materials' surfaces exhibit adequate osteoconductive properties.

The rabbit was chosen as an experimental animal model due to its low cost and ease of management, but most importantly for its high bone turnover, which is suggested to be two to three times faster than humans.[20] Thus, the chosen healing intervals in this study coincide with a timespan that covers both early and complete bone healing in humans.[21] The rabbit long bone model has been successfully used in various studies for initial evaluation of the bone/implant interface in relation to different surface treatments.[10,22,23]

In the current study, osseointegration was assessed using histological analysis of nondecalcified bone specimens containing the implant. Specimen preparation procedures proved to be very effective and reliable and did not involve any complications or loss of specimens. All sections were cut through the center of the implant with very little variations, so that histometric evaluation could be well standardized. The study focused on BIC as the main histometric parameter for the evaluation of the implant performance.

The present results demonstrated that fusion-sputtered zirconia implant surfaces had significantly higher BIC values compared with Ti and control zirconia implants at 4- and 8-week healing intervals; however, at 12 weeks, the BIC ratios of fusion-sputtered zirconia implants were marginally higher than those of Ti and failed to reach statistical significance. This may be attributed to a better initial healing process around the fusion-sputtered zirconia implant surface, which resulted in an accelerated osseointegration of the implants at an earlier time point. Osseointegration of Ti, on the other hand, has its onset at a later time, but with a slightly higher rate of bone apposition. These findings were in line with other animal studies, which recorded higher degrees of BIC in relation to zirconia implant surfaces, but failed to demonstrate statistical differences between structured zirconia and Ti implant surfaces.[9,11,17,24,25]

Histological results showed direct osseointegration between fusion-sputtered zirconia implant surfaces and the adjoining bone without interposition of any soft tissue. This was in contrast to Sennerby et al, who reported a presence of loose connective tissue layer separating bone tissue from zirconia surface.[17] In addition, fusion-sputtered zirconia implant surfaces achieved high mechanical stability in the host bone, confirmed by their high resistance to RTQ forces. Although not statistically significant, zirconia implants recorded marginally higher RTQ values than Ti implants over the studied healing periods. These results were in line with the study of Sennerby et al, who compared osseointegration of zirconia implants with either a machined or two different porous surfaces to Ti implants in the tibia and femur of 12 rabbits. RTQ results showed higher

Table 3 Bone density analysis of tested groups after 4, 8, and 12 weeks healing time (Mean ± SD)

Implant	BD-bt (%) 4 weeks[a]	BD-ot (%) 4 weeks[b]	BD-bt (%) 8 weeks[c]	BD-ot (%) 8 weeks[d]	BD-bt (%) 12 weeks[e]	BD-ot (%) 12 weeks[f]
Fusion-sputtered zirconia	45.9 ± 3.2A	49.9 ± 2.0A	54.3 ± 3.8A	60.5 ± 1.9A	65.2 ± 2.1A	69.4 ± 1.6A
Titanium	43.7 ± 2.8A	47.3 ± 2.2A	53.3 ± 1.3A	58.9 ± 3.5A	64.0 ± 2.3A	67.2 ± 1.2A
Control zirconia	38.3 ± 1.7	42.5 ± 1.8	46.5 ± 1.08	52.7 ± 3.6	60.4 ± 1.5	62.8 ± 1.9

BD-bt: bone density between implant threads; BD-ot: bone density outside implant threads. Means followed by the same capital letters do not differ statistically. [a]$F(2, 12) = 10.4$; $p = 0.002$. [b]$F(2, 12) = 17.39$; $p < 0.001$. [c]$F(2, 12) = 12$; $p < 0.001$. [d]$F(2, 12) = 8.52$; $p = 0.004$. [e]$F(2, 12) = 7.39$; $p = 0.008$. [f]$F(2, 12) = 20.2$; $p < 0.001$.

values for the zirconia implants with the two different porous structures than for the Ti implants; however, the results were not significant. The lowest values were found for the machined zirconia implants.[17]

The results of the RTQ measurements showed a substantial increase in RTQ values of fusion-sputtered zirconia implants and SLA Ti implants between 4 and 8 weeks, but there was no notable increase between 8 and 12 weeks, which can be attributed to the bone remodeling process around both implant types. In comparison with the published RTQ values in previous studies on surface-roughened zirconia implants (32.4 ± 17.0 after 4 weeks, 43.1 ± 19.0 after 8 weeks, 31.3 ± 12.8 after 12 weeks;[8] and 42.4 ± 15.1 after 4 weeks, 69.6 ± 25.1 after 8 weeks, 69.3 ± 24.2 after 12 weeks[26]), the current study showed higher RTQ values for fusion-sputtered zirconia implants, which highlights the beneficial effect of this novel surface treatment on the interfacial shear strength.

A possible clinical implication could be that a sputtered zirconia implant may be preferred for early or immediate loading situations because of better approximation of bone to the implant during early healing, possibly offering better stabilization compared to a Ti screw. That said, additional studies are recommended, as the sample size in this report was small.

Conclusion

Within the limitations of the present study and its sample size, fusion-sputtered zirconia implant surfaces demonstrated a degree of osseointegration and interfacial biomechanical properties comparable to sandblasted acid-etched titanium implants and substantially higher than as-sintered zirconia, which may improve the clinical performance of zirconia implants.

References

1. Albrektsson T, Branemark PI, Hansson HA, et al: Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 1981;52:155-170
2. Albrektsson T, Wennerberg A: Oral implant surfaces: Part 2—review focusing on clinical knowledge of different surfaces. Int J Prosthodont 2004;17:544-564
3. Badr NA, El Hadary AA: Hydroxyapatite-electroplated cp-titanium implant and its bone integration potentiality: an in vivo study. Implant Dent 2007;16:297-308
4. Jung RE, Holderegger C, Sailer I, et al: The effect of all-ceramic and porcelain-fused-to-metal restorations on marginal peri-implant soft tissue color: a randomized controlled clinical trial. Int J Periodontics Restorative Dent 2008;28:357-365
5. Glauser R, Sailer I, Wohlwend A, et al: Experimental zirconia abutments for implant-supported single-tooth restorations in esthetically demanding regions: 4-year results of a prospective clinical study. Int J Prosthodont 2004;17:285-290
6. Marinello CP, Meyenberg KH, Zitzmann N, et al: Single-tooth replacement: some clinical aspects. J Esthet Dent 1997;9:169-178
7. Piconi C, Maccauro G: Zirconia as a ceramic biomaterial. Biomat 1999;20:1-25
8. Gahlert M, Gudehus T, Eichhorn S, et al: Biomechanical and histomorphometric comparison between zirconia implants with varying surface textures and a titanium implant in the maxilla of miniature pigs. Clin Oral Implant Res 2007;18:662-668
9. Akagawa Y, Hosokawa R, Sato Y, et al: Comparison between freestanding and tooth-connected partially stabilized zirconia implants after two years' function in monkeys: a clinical and histologic study. J Prosthet Dent 1998;80:551-558
10. Hoffmann O, Angelov N, Gallez F, et al: The zirconia implant-bone interface: a preliminary histologic evaluation in rabbits. Int J Oral Maxillofac Implants 2008;23:691-695
11. Kohal RJ, Wolkewitz M, Hinze M, et al: Biomechanical and histological behavior of zirconia implants: an experiment in the rat. Clin Oral Implants Res 2009;20:333-339
12. Langhoff JD, Voelter K, Scharnweber D, et al: Comparison of chemically and pharmaceutically modified titanium and zirconia implant surfaces in dentistry: a study in sheep. Int J Oral Maxillofac Surg 2008;37:1125-1132
13. Rocchietta I, Fontana F, Addis A, et al: Surface-modified zirconia implants: tissue response in rabbits. Clin Oral Implants Res 2009;20:844-850
14. Att W, Takeuchi M, Suzuki T, et al: Enhanced osteoblast function on ultraviolet light-treated zirconia. Biomat 2009;30:1273-1280
15. Aboushelib MN, Kleverlaan CJ, Feilzer AJ: Selective infiltration-etching technique for a strong and durable bond of resin cements to zirconia-based materials. J Prosthet Dent 2007;98:379-388
16. Aboushelib MN, Feilzer AJ, Kleverlaan CJ: Bonding to zirconia using a new surface treatment. J Prosthodont 2010;19:340-346
17. Sennerby L, Dasmah A, Larsson B, et al: Bone tissue responses to surface-modified zirconia implants: A histomorphometric and removal torque study in the rabbit. Clin Implant Dent Relat Res 2005;7(Suppl 1):S13-20
18. Blaschke C, Volz U: Soft and hard tissue response to zirconium dioxide dental implants—a clinical study in man. Neuro Endocrinol Lett 2006;27(Suppl 1):69-72

19. Depprich R, Zipprich H, Ommerborn M, et al: Osseointegration of zirconia implants compared with titanium: an in vivo study. Head Face Med 2008;4:30
20. Robert WE, Turly PK, Brezniak N, et al: Implant: bone physiology and metabolism. J Calif Dent Assoc 1987;15:54-61
21. Matos MA, Arau'jo FP, Paixao FB: Histomorphometric evaluation of bone healing in rabbit fibular osteotomy model without fixation. J Orthop Surg Res 2008;3:1749-1799
22. Hsu SH, Liu BS, Lin WH, et al: Characterization and biocompatibility of a titanium dental implant with a laser irradiated and dual-acid etched surface. Biomed Mater Eng 2007;17:53-68
23. Bitschnau A, Alt V, Bohner F, et al: Comparison of new bone formation, implant integration, and biocompatibility between RGD-hydroxyapatite and pure hydroxyapatite coating for cementless joint prostheses—an experimental study in rabbits. J Biomed Mater Res B 2009;88:66-74
24. Dubruille JH, Viguier E, Le Naour G, et al: Evaluation of combinations of titanium, zirconia, and alumina implants with 2 bone fillers in the dog. Int J Oral Maxillofac Implants 1999;14:1-7
25. Kohal RJ, Weng D, Bachle M, et al: Loaded custom-made zirconia and titanium implants show similar osseointegration: an animal experiment. J Periodontol 2004;75:1262-1268
26. Gahlert M, Röhling S, Wieland M, et al: A comparison study of the osseointegration of zirconia and titanium dental implants. A biomechanical evaluation in the maxilla of pigs. Clin Implant Dent Relat Res 2010;12:297-305