Influence of Surface Nano-Roughness on Osseointegration of Zirconia Implants in Rabbit Femur Heads Using Selective Infiltration Etching Technique

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This study evaluates osseous healing of selective infiltration-etched (SIE) zirconia implants compared to as-sintered zirconia and titanium implants. Twenty implants of each group were inserted in 40 adult New Zealand white male rabbits. After 4 and 6 weeks, bone blocks containing the implants were retrieved, sectioned, and processed to evaluate bone-implant contact (BIC) and peri-implant bone density. SIE zirconia implants had significantly higher BIC and marginally higher bone density. The results suggest that selective infiltration-etched zirconia implant surface may improve implant osseointegration.

Key Words: selective-infiltration-etching, zirconia implants, peri-implant bone density, bone-implant contact, nano-technology, osseointegration

INTRODUCTION

Since their introduction, dental implants have been accepted as a predictable and reliable treatment modality for the rehabilitation of both partially and completely edentulous patients. Success of dental implants depends mainly on osseointegration of the implant into the host bone. Implant integration is greatly influenced by the properties of the implant surface (such as topography and chemistry). A wide range of surface modifications have been developed to improve and accelerate osseous healing of dental implants and to increase the strength and resistance of the bone-implant interface to long-term functional loading.[1–3]

Titanium and titanium alloys are the most commonly used dental implant materials, due to their excellent biocompatibility. Their high success and survival rates have been confirmed in different applications in the oral cavity. However, the main disadvantage of titanium implants is its dark grayish color that may lead to esthetic impairment with unfavorable soft tissue response or thin gingival biotype.[4] Zirconia offers a promising alternative as a dental implant material, due to its tooth-like color and ability to transmit light, which makes it superior in esthetically critical areas of the oral cavity. Zirconia also possesses excellent mechanical properties represented mainly by high fracture resistance and flexural strength.[5,6] This is mostly due to the transformation of zirconia tetragonal particles into monoclinic ones, accompanied by a simultaneous 4% expansion in volume, which interrupts the propagation of a crack or a flaw and prevents fracture of the material under stress.[7,8] In addition, it exhibits high values of bending strength and a relatively low modulus of elasticity indicating a higher elastic deformation capability.[9] Zirconia also has good chemical and dimensional stability and excellent resistance to corrosion and wear.[10] Zirconia is considered to be a highly biocompatible

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DOI: 10.1563/AAID-JOI-D-11-00075

material. Subcutaneously implanted zirconia disks were completely encapsulated by a thin layer of fibrous connective tissue with only minor inflammatory cell infiltrate.[11] In other studies, the material did not cause any toxic, immunologic, or carcinogenic effects[12] and had no oncogenic effect in vitro.[13] Biocompatibility of zirconia implants has also been confirmed with direct bone apposition to the implant surface.[9,14]

Various animal studies and numerous case reports demonstrated that osseointegration of zirconia implants is similar or even superior to that of titanium implants.[15–18] Fabrication of zirconia rods usually results in a relatively smooth surface, and combined with the bio-inert nature of the material, renders the surface of the resulting implants to be dense and nonretentive. Different approaches are being used in an effort to improve surface properties of zirconia as airborne-particle abrasion, acid etching with hydrochloric or hydrofluoric acids, plasma spraying,[10,18] aggregation of bioactive materials such as hydroxyapatite,[17] and more recently, ultraviolet radiation has been used to increase the hydrophilic properties of the zirconia implants.[19]

Selective infiltration-etching (SIE) technique is a new surface treatment used to transform the surface of zirconia into a highly retentive nanoporous surface.[20] In this technique, the surface of zirconia is coated with a special infiltration glass and heated above its glass transition temperature. The molten glass diffuses between the grain boundaries of zirconia, exerting surface tension and capillary forces which results in sliding, splitting, and rearrangement of the surface grains. After cooling to room temperature, the glass is dissolved in an acidic bath, exposing the newly created nano-scale intergrain surface porosities.[21,22] This surface treatment is selective as it involves only the surface grains, and the architecture and distribution of the created porosities could be controlled by changing the composition of the glass together with the heat and time parameters. The technique was successfully used to increase bond strength of resin cement to zirconia restorations[21–23]; nevertheless, it could also be applied to improve osseointegration of zirconia implants.[24] The aim of this animal study was to evaluate osseous healing of selective infiltration-etched zirconia implants in comparison with as-sintered zirconia and titanium implants.

Materials and Methods

Preparation of the specimens

Forty zirconia implants were fabricated using compression molding technique with 50 μm zirconia powder (E grade 3 mol Y-TZP, Toso Inc, Tokyo, Japan). The pressed implants were sintered at 1350°C for 6 hours. The final implants were identical in shape and dimension (8 mm × 3.7 mm) to commercially available titanium implants (SLA, Tapered SP MTX, Zimmer Dental, Carlsbad, Calif) in order to standardize insertion and handling procedure. Half of the prepared zirconia implants received selective infiltration-etching surface treatment through coating with infiltration glass, thermal heating, and washing off glass residues. Step-by-step details are described elsewhere.[21] The surface roughness of the implants was measured at the flat end of the abutment head using contact profilometry (Marsurf PS1, Mahr GM6H, Göttingen, Germany). Surface topography of both selective infiltration-etched and as-sintered zirconia implants were photographed at different magnifications using scanning electron microscopy (JSM-5300, Jeol, Japan) following specimens gold coating using a sputter device (JFC-1100E ion sputtering device, Jeol, Tokyo, Japan).

Experimental animals

Forty adult male New Zealand white rabbits (8 months old) weighing approximately 5.0–5.5 kg were included in this study. The study was approved by the Ethics Committee of Alexandria University. The animals were housed in individual stainless-steel cages in an animal room. Temperature was maintained at 24 ± 4°C and relative humidity at about 50%–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 before surgery.

Surgical procedures

All surgeries were performed under sterile conditions in a veterinary operating theater by an expert surgeon. The animals were operated under general anesthetic induced by intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). In the areas to be 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 femur heads on both sides were surgically exposed via a skin incision, and the muscles were dissected to allow elevation of the periosteum and exposure of bone surface. The implant site (right and left femur heads) was prepared using sequential water cooling surgical drills with increasing diameter under profuse irrigation with sterile saline solution. The implants were inserted and threaded to bone level (Zimmer surgical kit, Zimmer Dental) (Figure 1a through c). 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 until full recovery.

Twenty rabbits received selective infiltration-etched and as-sintered zirconia implants in contralateral femur heads, while the other 20 animals received control titanium implants for a 4- and 6-week healing interval (10 animals/implant type/test interval). 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, intramuscularly) and a 7-day course of a broad-spectrum antibiotic (amoxylline 5 mg/kg, intramuscularly) was administered for infection control.

After a healing period of 4 and 6 weeks, bone blocks containing the implants were dissected from the animals after injection of an overdose of a general anesthetic. Unnecessary fragments of bone and soft tissue were removed, and the specimens were prepared for histologic investigation.

Histologic analysis

Bone blocks were immediately fixed in 4% buffered formaldehyde, and 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 precision cutting machine (Micracut 150 precision cutter, Metkon, Bursa, Turkey) followed by grinding and polishing with 800-grit silicon carbide paper. At least 4 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 1 expert examiner, who was blind to the grouping of the specimens, using an image analysis software system (version 3.3, Olympus CellM & CellR, Olympus Soft Imaging Solutions GmhH, Munster, Germany). 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 length. Bone density within and outside the implant threads were calculated using the image contrast feature of the used software under higher magnifications. Percent of mature bone (stained red) was calculated against percent of soft tissue (stained blue) 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) (Figure 2a and b).

Statistical analysis

Examiner reliability for the histometric analysis was crosschecked 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 of the measured parameters. Data were expressed as mean and standard deviation. One-way analysis of variance with Bonferroni post hoc test was used for statistical analysis ($\alpha = .05$). All calculations were performed using SPSS version 15.0 software (SPSS, Chicago, Ill).

Results

All animals survived the treatment with uneventful healing period and were available for evaluation.

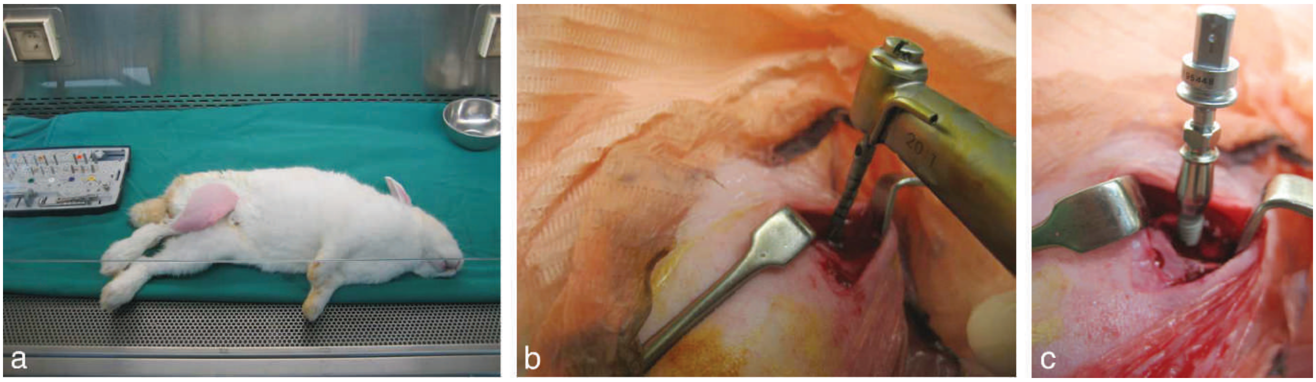


Figure 1. (a) Anesthetized animal on operating table behind airflow barrier. (b) Preparation of implant site using sequential drills following exposure of femur head. (c) Insertion of titanium implant at the prepared implant site.

Only one animal was excluded from the study because of hematoma formation at the experimental site, the animal was accordingly replaced. All implants appeared to be osseointegrated and were clinically stable upon retrieval.

Scanning electron microscopy

Scanning electron microscope images revealed that selective infiltration-etched implants demonstrated intergrain surface porosity compared to the dense nonretentive surface of the as-sintered zirconia implants (Figure 3a and b). The surface of the used titanium implant demonstrated typical features of sand-blasted acid-etched surface treatment.

Histologic results

At 4 weeks of healing time, immature bone was present in direct apposition to all the studied implant surfaces without interposition of any soft tissue, indicating direct osseous integration of all implants (Figure 4). Woven bone and osteoid tissue were also evident, confirming an ongoing process of new bone formation. After 6 weeks, mature mineralized lamellar bone was observed directly on and along the entire surfaces of all analyzed implants, with no apparent histologic differences (Figure 5).

Histometric analysis

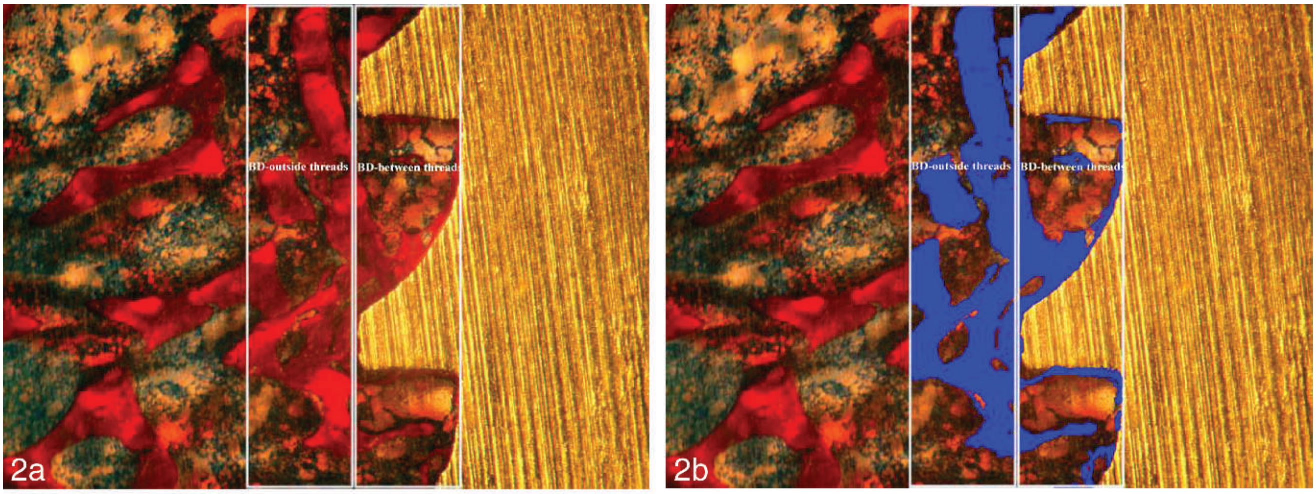
Following a 4-week healing period SIE zirconia implants demonstrated significantly greater BIC compared to that of as-sintered zirconia and titanium implants. The mean BIC for SIE zirconia implants was 65.38 $\pm$ 5.7 compared to 53.30 $\pm$ 4.2 for the as-sintered zirconia and 56.93 $\pm$ 3.9 for the titanium implants. This difference was found to be statistically significant ($P < .05$). After 6 weeks of healing period, BIC for SIE zirconia implant surface remained statistically higher ($P < .05$) than that of as-sintered zirconia and titanium implants, 75.01 $\pm$ 5.1, 62.14 $\pm$ 2.8, and 68.31 $\pm$ 4.2, respectively.

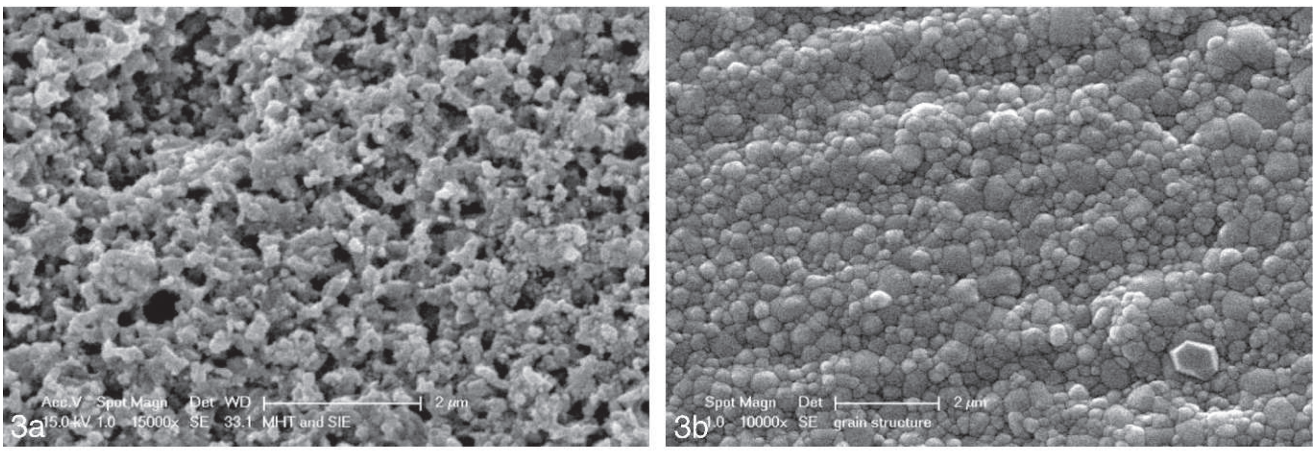
There were no significant differences in the measured bone density within or outside the implant threads observed at both tested intervals between all tested implants. However, selective-infiltration-etched zirconia implants had marginal higher bone density within and outside the threads compared to as-sintered zirconia and titanium implants. The previous data are summarized in the Table.

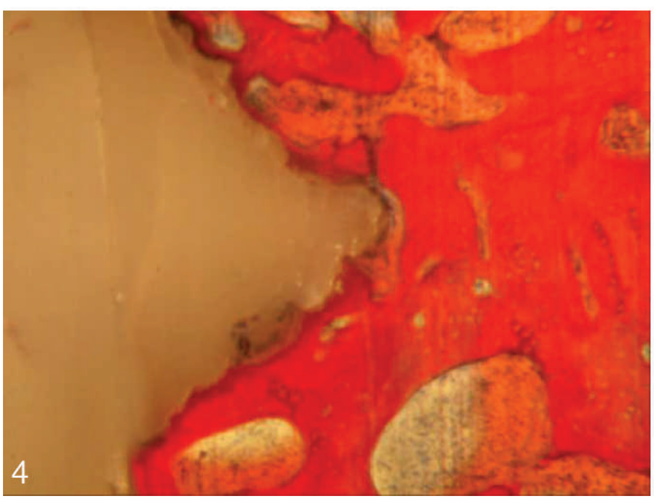
Discussion

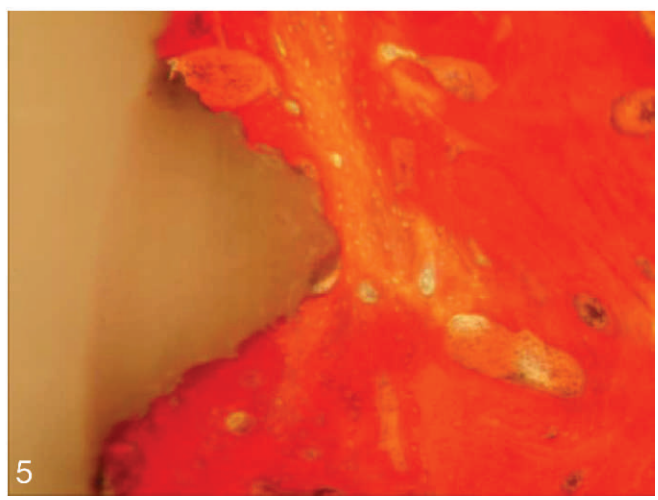
Based on the findings of the present study, selective infiltration-etched surface treatment enhanced osseointegration as evident by histometric analysis, which demonstrated higher percentage of BIC compared to as-sintered zirconia and titanium implants. The nano-porous surface characteristics improved osseous healing at the bone-implant interface, demonstrating the beneficial effect of roughening zirconia implant surface, which was in line with previous studies comparing osseointegration of zirconia and titanium implants.[14,25]

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 2–3 times faster than humans[26]; thus the chosen healing interval in this study coincides with a period that covers both early and complete bone healing in









Figures 2–5. Figure 2. (a) Histometric analysis of bone density between and outside implant threads using a representative digital image of a titanium implant in host bone after 6 weeks of implantation, showing mature bone in direct contact with the implant surface (stained red), ×110. (b) Previous image after application of color contrast image analysis feature (mature bone in blue color). **Figure 3.** (a) Scanning electron microscopic micrograph of nano-porous surface of selective infiltration-etched zirconia implant, demonstrating intergrain surface porosity, ×15 000. (b) Scanning electron microscopic micrograph, demonstrating typical grain structure of as-sintered zirconia implants, ×10 000. **Figure 4.** Micrograph of selective infiltration-etched zirconia implant, after 4 weeks of implantation, showing newly formed bone in direct contact with the implant surface, (Stevenel's Blue and Van Gieson's stain ×220). **Figure 5.** Micrograph of selective infiltration-etched zirconia implant, after 6 weeks of implantation, showing mature mineralized lamellar bone formed directly on the implant surface, (Stevenel's Blue and Van Gieson's Stain ×220).

TABLE

Histometric analysis of tested groups after 4 and 6 weeks of healing time (mean ± SD)*

	BIC (%)	BIC (%)	BD-bt (%)	BD-bt (%)	BD-ot (%)	BD-ot (%)
Implant	4 wk	6 wk	4 wk	6 wk	4 wk	6 wk
SIE zirconia	65.38 ± 5.7†	75.01 ± 5.1‡	47 ± 4.2	54 ± 5.6	51 ± 6.2	52 ± 4.3
As-sintered zirconia	53.30 ± 4.2	62.14 ± 2.8	38 ± 3.3	41.8 ± 2.1	42 ± 5.0	48 ± 3.8
Titanium	56.93 ± 3.9	68.31 ± 4.2	40.8 ± 2.5	41 ± 3.7	44 ± 3.6	49 ±1.6

*BIC indicates bone implant contact; BD-bt, bone density between implant threads; BD-ot, bone density outside implant threads; SIE, selective infiltration-etched.

†Significant difference in comparison with 4 weeks of as-sintered zirconia and titanium implants ($P = .002$).

‡Significant difference in comparison with 6 weeks of as-sintered zirconia and titanium implants ($P = .003$).

humans.[27] 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.[15,28,29]

Color contrast histometric analysis was performed in this study to calculate bone density within and outside the implant threads. This feature of the used software ensured accurate calculation of the percent of mature bone (red stain) in the required field in comparison to soft tissue and immature bone, which enabled evaluation of the mineralization process at different time intervals. Backscatter scanning electron microscope images, micro computed tomography imaging, and digital x-ray imaging are suitable alternatives that can be used to evaluate bone implant interface, each with its own advantages and drawbacks.

Zirconia bioceramics show excellent biocompatibility, high mechanical properties, good chemical and dimensional stability, in addition to their superior tooth like color, which potentially makes them a suitable material for fabrication of dental implants. Owing to a phenomenon known as transformation toughening, zirconia has a higher fracture resistance than previously available ceramics, which makes it less sensitive to stress concentrations.[6,7] Moreover, thanks to its high flexure strength, thin parts of restorations (threads feature of implants or abutment walls) could be fabricated without the risk of fracture.[30,31] On the other hand, zirconia has a hard, dense, and chemically inert surface[5] that ensures its chemical stability and long-term performance under the tough conditions of the oral environment, but is not optimal for osseointegration. Different surface roughing procedures were studied in order to enhance surface characteristics of zirconia implants, thus improving osseointegration.[10,18,32] Airborne particle abrasion and surface grinding resulted in structural damage, material loss, grain pullout, and induction of microcracks, causing zirconia to crack under functional loading.[30,33,34]

A novel surface roughening procedure, selective infiltration-etching, was investigated in this study. The technique is used to create a nano-porous surface through application of a specific infiltration glass under controlled heat, resulting in micromovements of surface grains without the need to exert any external stresses. Through reorganization, sliding, and splitting of the surface grains, it was possible to create nano-scale surface porosities without the creation of structural defects.[20–22] It does not induce surface or subsurface damage observed with airborne particle abrasion and other roughening techniques.[33] The technique also has an advantage over surface-coated implants in that it eliminates chances of delamination and cohesive failure between implant and coating material.[35]

Clinical success of dental implants depends on the osseointegration of the implant in the host bone, which is directly affected by the implant surface texture and properties. Several animals and clinical studies reported successful osseointegration of roughened zirconia implant surfaces that was comparable to titanium implants.[14,36,37] In the present study, selective infiltration-etched zirconia implants demonstrated significantly higher bone-implant contact values when compared to as-sintered zirconia and titanium implants. This finding was in line with other animal studies, which recorded higher degrees of bone-implant contact in relation to zirconia implant surfaces, but failed to demonstrate statistical differences between structured zirconia and titanium implant surfaces.[14,25,38]

In this study, the measured BIC values for zirconia implant surfaces were higher than those measured in a similar study[39] where the authors reported BIC of 68.6 and 64.5 for their studied zirconia surfaces after a 6-week healing interval in the femur of rabbits. This difference may be attributed to the surface properties of selective infiltration-etched implants in term of nano-roughness, capillary forces, and wetting properties. In the present study, all studied implants showed direct osseointegration between the implant surface and the adjoining bone without interposition of any soft tissue. On the other hand, Sennerby et al[38] reported the presence of loose connective tissue layer separating bone tissue from zirconia surface. Bone density measurements within and outside implant threads were slightly higher for selective infiltration-etched zirconia implants after 4 and 6 weeks of healing time, which also corresponds to higher degree of osseointegration. This confirms the finding that surface microroughness and architecture is a key factor influencing osseointegration of the tested implant materials.

Conclusion

Within the limitations of the present study and its sample size, selective infiltration-etched zirconia implants demonstrated significantly higher degree of osseointegration in rabbit femur heads, as measured by BIC, compared to as-sintered zirconia and titanium implants, which may improve clinical performance of zirconia implants.

Abbreviations

BIC: bone-implant contact
SIE: selective-infiltration-etched

Acknowledgment

This study was supported by a research grant from Science and Technology Department Fund (Reintegration grant ID-489) awarded by the Egyptian Academy of Scientific Research.

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