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Mechanical, histological and histomorphometric evaluation of modified by femtosecond laser zirconia implants versus titanium implants. An experimental study in dogs at three months

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ABSTRACT

Aim The present study was aimed at quantifying implant´s stability and elemental composition by Periotest® and evaluating bone to implant contact (BIC) and crestal bone loss of modified by femtosecond laser zirconia and titanium implants.
Materials and methods Forty-eight implants were divided into 2 groups: titanium (control) and modified by femtosecond laser zirconia (test) and then inserted in 6 American Foxhound dogs. Primary stability and secondary stability were measured by Periotest, BIC was evaluated by histomorphometry at 1 and 3 months, elemental composition of the surrounding bone in both groups after 1 and 3 months was assessed.
Results Differences between groups regarding primary stability and secondary stability were not significant ($p>0.05$). The Carbon ratio at zirconia (12.529%) was significantly lower ($p<0.05$) at 1 month than titanium (15.776%). The highest Calcium rates were found on titanium (16.248% and 17.007%), but the differences between groups were not significant. No statistically significant differences ($p>0.05$) regarding BIC for titanium vs modified zirconia were observed. Crestal bone loss at 3 months was significantly ($p<0.05$) lower (0.07 ± 0.34 mm) in titanium group than in zirconia (1.25 ± 1.73 mm).
Conclusion Surface treatment by using femtosecond laser equalizes osseointegration of zirconia implants.

KEYWORDS Bone to implant contact; Crestal bone loss; Dental implants; Implant stability; Titanium; Zirconia.

INTRODUCTION

Despite the extensive use worldwide and the well established long term success of titanium dental implants (1-4), there are some disadvantages that should be considered. Indeed, the possibility of increased sensitivity reactions to titanium, titanium allergy (5-7), gingival retraction or gingival translucency with the consequent risk, especially in aesthetic zones of the maxilla and in thin gingival biotypes, that the grey color of titanium implants may be visible (8-10), should be taken into account in order to overcome these limits.
For these reasons, it has been suggested the use of Yttria stabilized polycrystalline tetragonal zirconia (Y-TZP) implants as an alternative to titanium implants. Zirconia implants have been reported to show excellent bone response, minimum inflammatory reactions, biocompatibility, excellent optical and aesthetic properties, low bacterial adherence to their surface, high fracture and compression resistance (11-19). Zirconia dioxide is resistant to chemical treatments, especially to acid etching (20), therefore, commercial zirconia implant systems are treated by superficial sandblasting. Physical techniques of microtexturing may cause random geometries and superficial roughness values at micrometric and nanometric levels, however, these treatments are no clean processes (21). Another disadvantage of these microtexturing techniques is the difficulty on their reproducibility because the final result of the process cannot be controlled (22); and sometimes it is necessary to use conductive materials and special vacuum conditions.
One promising technique for surface treatment in order to obtain a precise control of texture is microtexturing by means of laser (23). From the 60´s to the present day laser technology has much developed. In the recent years, one of the development lines has been the production of high intensity pulse source, which produces extremely

short pulses, lower than picoseconds. This pulse laser, amplified to obtain energies of milijoule (mJ) and conveniently focalized to material surface, allows surface ablation with extreme precision and reproducibility, and produces lighter unwanted injuries to the adjacent material than any other thermal source, chemical or mechanical process. The most important advantages of this laser processing applied to zirconia are as follows.

- It is a clean process.
- It produces minimal thermal injuries on the material surface.
- It can be applied on any surface.
- It does not require any special environment.
- It enables the achievement of precision and high quality geometry.
- It is reproducible (24).

The aims of this study were: the following.

- To quantify implant's stability of modified by femtosecond laser zirconia implants and titanium implants by Periotest® at 1 and 3 months.
- To quantify elemental composition of bone in modified by femtosecond laser zirconia implants and titanium implants at 1 and 3 months.
- To measure and compare BIC of sandblasted zirconia implants treated with femtosecond laser and titanium implants by histological and histomorphometric analysis using scanning electron microscope at 1 and 3 months on American Foxhounds.
- To measure and compare crestal bone loss around sandblasted zirconia implants, treated with femtosecond laser, and titanium implants by histological analysis at 4 and 12 weeks on American Foxhound model.

MATHERIALS AND METHODS

Six female American Foxhounds of one year of age, each weighing 14-15 kg were used in the present study. The Ethics Committee for Animal Research at the University of Murcia, Spain, approved the study protocol on May 12th 2010. For all the animals the Royal Decree (RD) 1201/2005 of October 10th, on protection of animals used for experimental and other scientific purposes was followed, as well as LAW 32/2007 of November 7th, for the care given to animals at the farm, transport, testing and sacrifice. Furthermore, the project followed 2010/63/UE directive of the European parliament and of the council of September 22nd 2010 on protection of animals used for scientific purposes. The animals were fed a daily pellet diet. Clinical examination determined that the dogs were in good general health.

Surgical procedure

The animals were pre-anesthetized with Acepromazine 0.2–1.5% mg/kg 10 min before administering Butorphanol (0.2 mg/kg) and Medetomidine (7 mg/kg). The mixture was injected intramuscularly in the femoral quadriceps. An intravenous catheter was inserted in the cephalic vein and Propofol was infused at a slow, constant rate of 0.4 mg/kg/min. Local infiltrative anesthesia was administered at the surgical sites. These procedures were carried out under the supervision of a veterinary surgeon. Bilateral mandibular tooth extractions (P2, P3, P4 and M1) were performed. The teeth were sectioned in a bucco-lingual direction at the bifurcation using a tungsten carbide bur; the roots were individually extracted using a periotome and forceps, without damaging the bone walls (Fig. 1). Wound closure was carried out using single non resorbable sutures (3-0 TB-15. Lorca Marín, Ref. 55346).

During the first week after surgery, the animals received antibiotics and analgesics: Amoxicillin (500 mg, twice daily) and Ibuprofen 600 mg (three times a day) systemically. Sutures were removed after 2 weeks. The dogs were fed a soft diet for 14 days after the sutures were removed.

Implants were placed after a healing period of 2 months (Fig. 2). After crestal incision, a full thickness flap was raised and each site was prepared following the protocol recommended by the implant manufacturer (Bredent medical® GMBH & Co. KG, Senden, Germany), preparing a bed having a diameter of 4 mm and 10 mm in length. Each mandible received 4 cylindrical screw implants, all with the same dimensions in the intraosseous portion (Fig. 3). After the suture was positioned, an x ray of implants as well as a check of primary stability of the implants by Periotest® was undertaken (Fig. 4). The secondary stability of implants was measured on the day of the animal's sacrifice.

FIGG. 1 A: 2nd, 3rd, 4th mandibular premolar and 1rst mandibular molar. B: hemisection of tooth. C: detail of an extraction socket.

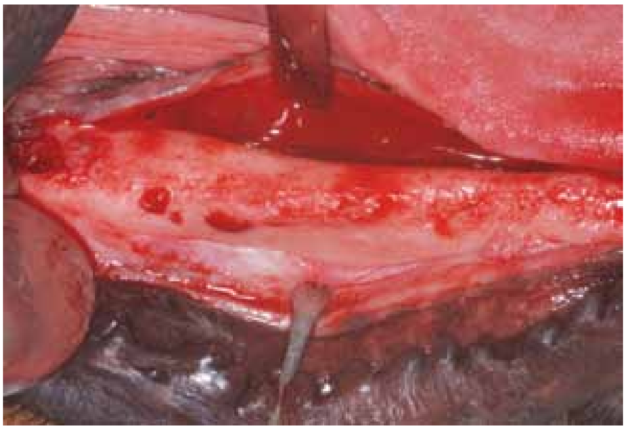


FIG. 2 Bone detail after two months of healing: a complete bone healing could be observed.

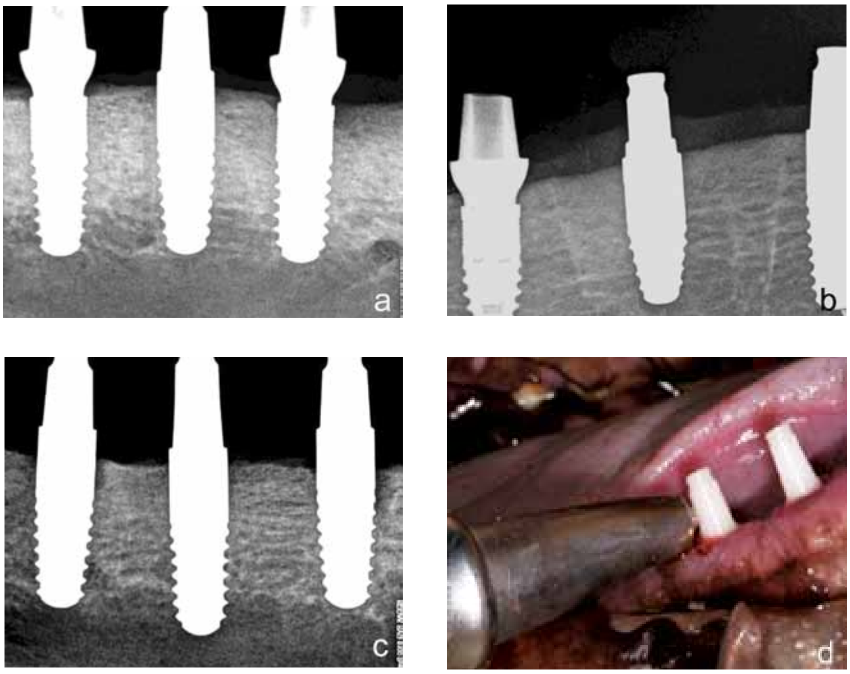
Implants

Forty-eight 4x10 mm implants were used, randomly divided into 2 groups: 24 titanium Blue-sky® implants (Bredent medical® GMBH & Co. KG, Senden, Germany) (control group); 24 White SKY® sandblasted zirconia implants (Bredent medical® GMBH & Co. KG, Senden, Germany) treated with femtosecond laser pulses to create 30 μm wide, 70 μm pitch length microgrooves over the entire intraosseous surface (test group).

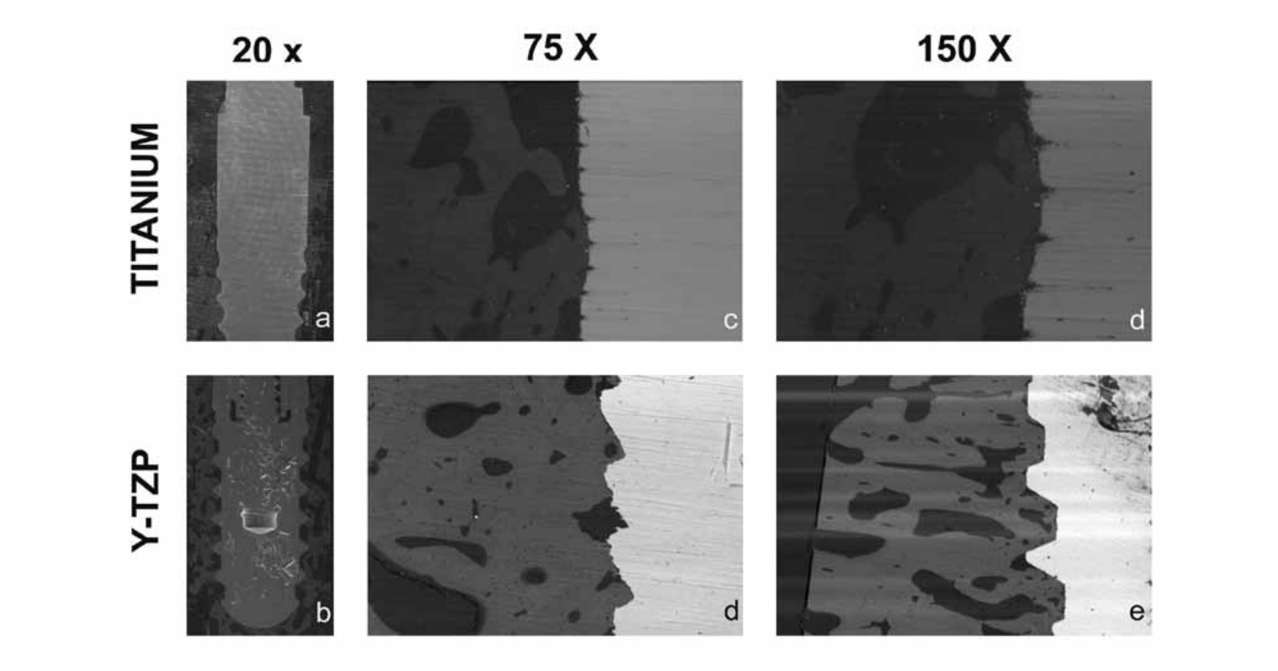
Histological preparation

Three animals were sacrificed for each time period by an infusion of sodium pentothal (Abbott Laboratories, Chicago, IL, USA) through the carotid artery with a fixative containing a mixture of 5% glutaraldehyde and 4% formaldehid. The veterinarians confirmed the dog's

FIGG. 3 Implant insertion. A: detail of site preparation using a drill with stops. B: parallel verification with parallelization pins. C: detail of Blue-Sky® and White-Sky® implants (Bredent medical® GMBH & Co. KG, Senden, Germany) insertion.



FIGG. 4 A, B, C: Postoperative radiographic images. D: clinical evaluation of primary stability by Periotest®.



FIGG. 5 Scanning electron microscope images of titanium and Y-TZP implants x20 (A, B) x75 (C, D) and x150 (E, F) magnification.

death and immediately proceeded to the dissection of the mandible. Jaws were dissected from each animal and each study area was extracted using a high speed diamond bur.
The samples were fixed in formalin and dehydrated in a graded series of ethanol for 15 min each and dried with acetone at 30%, 50%, 70% and 90% for 15 minutes each, then 100% acetone for 30 minutes.
The samples were then embedded in methylmethacrylate (Technovit 7100®, Heraeus Kulzer, Wehrheim, Germany) and processed for scanning electron microscope (SEM) analysis (Fig. 5). Using a micro-cut diamond (Exakt-Apparatebau, Norderstedt, Germany), they were cut in vestibulo-lingual direction, at 100 µm-thickness along the axis of each implant. The sections were grinded to 50-80 µm thickness using polishing techniques with extrafine paper discs with 2000 grain granulometry. The other halves of implants and surrounding bone were stained with toluidine blue for the histological analysis (Fig. 6).

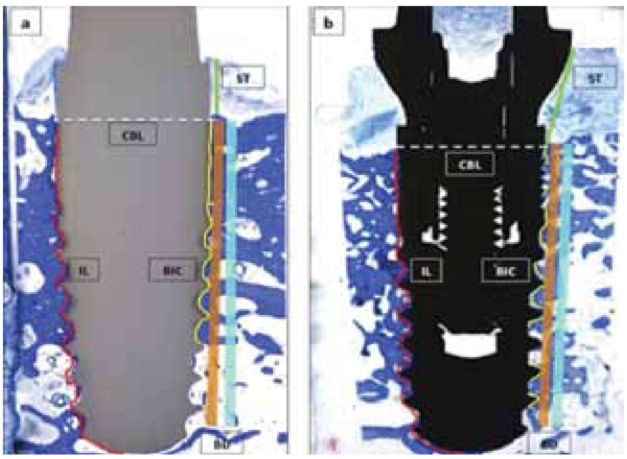


FIG. 6 Optical microscope images with analysis parameters (CBL: crestal bone level; BIC: bone-to-implant-contact; IL: implant length).

Statistical analysis

Calculations were performed using SPSS 15 (Chicago, Illinois, USA), licensed from the University of Murcia,

GROUP	N	MEAN	SD	P-VALUE
Zir Day 0	12	-6.167	0.7178	P>0.05
Ti Day 0	12	-5.250	0.866	P>0.05

TABLE 1 Initial primary stability (SD: standard deviation; level of significance $p<0.05$).

GROUP	N	MEAN	SD	P-VALUE
Zir 1 month	12	-6.833	0.7177	P>0.05
Ti 1 month	12	-5.750	0.7538	P>0.05
Zir 3 months	12	-6.583	1.505	P>0.05
Ti 3 months	12	-5.750	1.422	P>0.05

TABLE 2 Secondary stability at one and three months (SD: standard deviation; level of significance $p<0.05$).

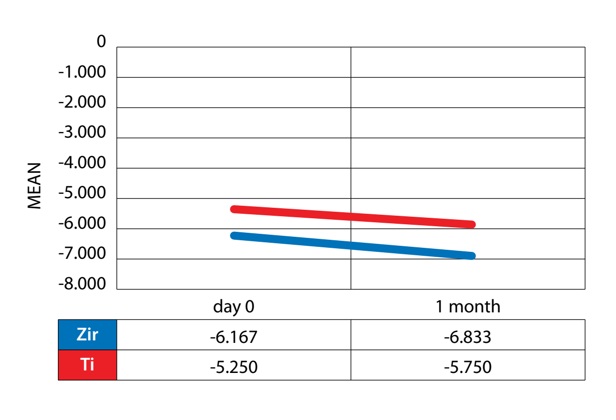


FIG. 7 Initial primary stability and secondary stability at 1 month.

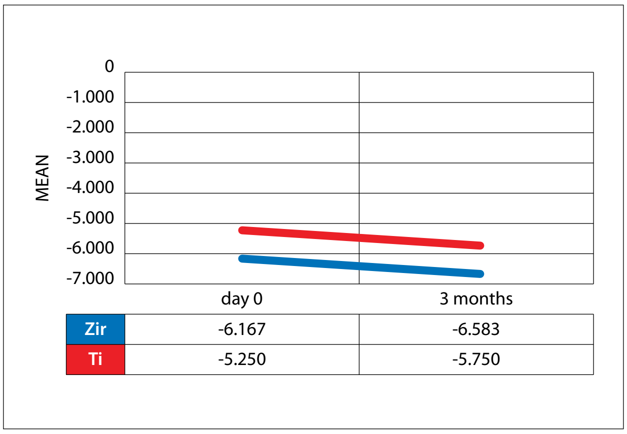


FIG. 8 Initial primary stability and secondary stability at 3 months.

	%C Ti 1 MONTH	%C Ti 3 MONTHS	%C Zir 1 MONTHS	%C Zir 3 MONTHS	%O Ti 1 MONTH	%O Ti 3 MONTHS	%O Zir 1 MONTH	%O Zir 3 MONTHS
N	12	12	12	12	12	12	12	12
Mean	15.776	11.686	12.529	13.485	12.277	14.978	12.347	12.508
SD	0.3824	0.6862	0.5289	0.8032	0.4546	0.4698	0.4574	0.3868
P-value	P<0.05*	P<0.05*	P<0.05*	P<0.05*	P>0.05	P<0.05*	P>0.05	P<0.05*

TABLE 3 Elemental analysis of C and O elements (SD: standard deviation; level of significance p<0.05*).

	%P Ti 1 MONTH	%P Ti 3 MONTHS	%P Zir 1 MONTHS	%P Zir 3 MONTHS	%CA Ti 1 MONTH	%CA Ti 3 MONTHS	%CA Zir 1 MONTH	%CA Zir 3 MONTHS
N	12	12	12	12	12	12	12	12
Mean	3.768	4.2525	3.853	3.911	16.248	17.007	15.967	16.348
SD	0.1937	0.3709	0.4579	0.2994	0.4426	0.4479	0.8780	1.093
P-value	P>0.05	P<0.05*	P>0.05	P<0.05*	P>0.05	P>0.05	P>0.05	P>0.05

TABLE 4 Elemental analysis of P and Ca elements (SD: standard deviation; level of significance p<0.05*).

setting a value of p<0.05. A descriptive statistic method was used. In addition, ANOVA test was used to compare differences between groups. Bonferroni test was also performed to compare mean averages between groups.

RESULTS

Primary and secondary implant stability

Regarding primary stability, the mean of Periotest® values were lower for modified zirconia implants (-6.176) than titanium implants (-5.250). The highest value (-5.250) was found at day 0 in the titanium group, whilst the lowest (-6.833) value in the modified zirconia group at 1 month (Table 1, 2).
In addition, it was observed that the Periotest values (PTVs) were lower at 1 month than at day 0 in both zirconia and titanium groups (Fig. 7). On the contrary, PTVs at 3 months increased or did not show any variations when compared with the first month (Fig. 7, 8) in both groups.
No significant differences (p>0.05) between groups were observed regarding primary stability (Table 1) and secondary stability at 1 and 3 months (Table 2) (p>0.05).

Elemental analysis

Significant differences (p<0.05) were found regarding the presence of Carbon element between groups at 1 month and 3 months. Specifically, the percentage of Carbon element in the zirconia group (12.529%) was significantly lower than in titanium group (15.776) at 1 month, but significantly higher at 3 months (Table 3).

On the contrary, regarding the Oxygen element, there were no significant differences between groups at 1 month (p>0.05), although Oxygen was higher in the zirconia group (12.347). On the other hand, at 3 months, the mean percentage of Oxygen was significantly lower (p<0.05) in the zirconia group (Table 3).
Regarding Phosphorum, the differences between groups were not significant (p>0.05) at 1 month (Ti: 3.768; Zir: 3.853), whilst at 3 months the percentage of Phosphorum was significantly higher (p<0.005) in the titanium group (Table 4).
Finally, no significant differences (p>0.05) between groups were seen regarding calcium at both periods of time, although, the highest percentages of calcium were found in the titanium group at 1 and 3 months (Table 4).

Bone to implant contact (BIC)

In titanium implants BIC was 51.36% ± 12.03% and 61.73% ± 16.27% at 1 and 3 months, respectively (Table 5).
In zirconia implants modified by femtosecond laser, BIC was 44.68% ± 17.66% during the first month and 47.94% ± 16.15% at 3 months (Table 5).
Notwithstanding the increase of BIC values in both groups (Fig. 9), no statistically significant differences (p>0.05) between the titanium implant group and the group of zirconia implants modified by femtosecond laser (Table 5) were detected.

Crestal bone loss

Crestal bone loss was higher in the first month compared with the third month in both groups (Fig. 10); specifically, in titanium implants the mean of crestal bone was 0,77 ± 0.69 mm, whilst in Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) group it was 0.45 mm ± 0.37 mm (Table 6). On the other hand, at 3 months crestal bone loss was smaller than at 1 month in both groups; in

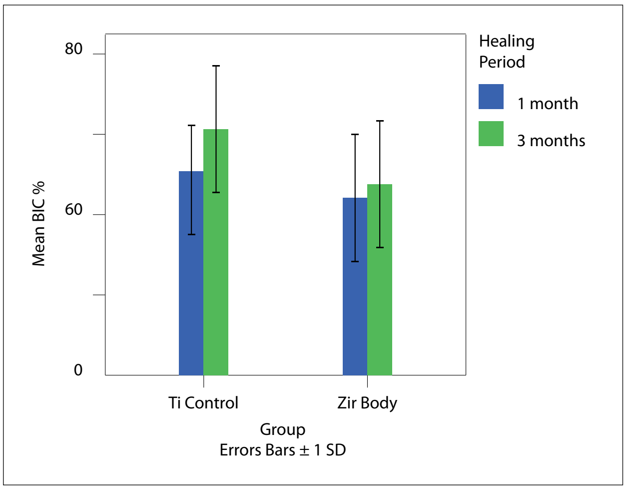


FIG. 9 Mean± standard deviation of BIC % for both groups and time periods.

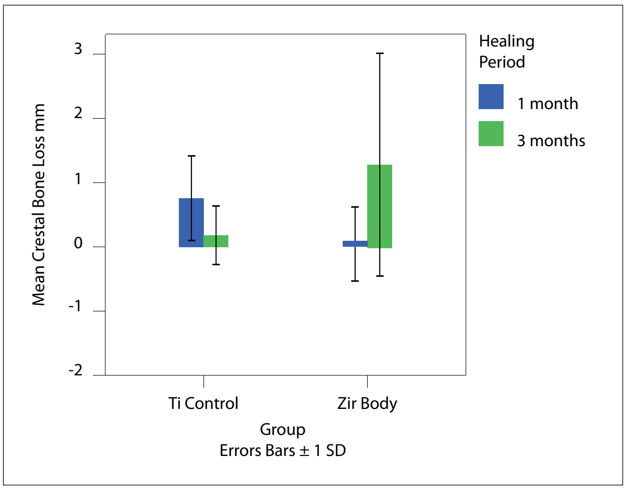


FIG. 10 Mean± standard deviation of crestal bone loss for both groups and time periods.

GROUP	HEALING PERIOD	N	MINIMUM	MAXIMUM	MEAN	SD	P-VALUE
TI_Control	1 month	12	-0.11	1.80	0.7693	0.69107	P>0.05
TI_Control	3 months	12	-0.34	0.59	0.0728	0.34128	P<0.05*
Z-Body	1 month	12	-0.67	1.02	0.0135	0.56662	P>0.05
Z-Body	3 months	12	-0.78	3.50	1.2532	1.73168	P<0.05*

TAB. 5 BIC contact of zirconium and titanium groups at both time periods (level of significance p<0.05*).

GROUP	HEALING PERIOD	N	MINIMUM	MAXIMUM	MEAN	SD	P-VALUE
TI_Control	1 month	12	-0.11	1.80	0.7693	0.69107	P>0.05
TI_Control	3 months	12	-0.34	0.59	0.0728	0.34128	P<0.05*
Z-Body	1 month	12	-0.67	1.02	0.0135	0.56662	P>0.05
Z-Body	3 months	12	-0.78	3.50	1.2532	1.73168	P<0.05*

TAB. 6 Crestal bone loss of zirconium and titanium groups at both time periods (level of significance p<0.05*).

particular, the titanium group showed lower values (0.07 ± 0.34 mm) than Y-TZP group (1.25 ± 1.73 mm) (Table 6). No significant differences were found when comparing crestal bone loss at 1 month between titanium and zirconia groups; although zirconia implants showed lower values of crestal bone loss. On the other hand, after comparing both groups at 3 months, crestal bone loss was significantly lower in titanium implants (Table 6).

DISCUSSION

Some factors that may affect primary stability are the design and the surface of dental implants. Threaded implant design minimizes micromovement during function, preserving implant stability, and increases surface area, giving a higher mechanical interlock with the surrounding bone (primary osseointegration) and favoring a higher BIC (25). Surface characteristics of implants also influence osseointegration. A rough surface positively influences osseointegration, promoting favorable cellular responses and adequate interactions with implant surface (26). A rough implant surface improves primary stability due to the increase of the contact area of the implant with the surrounding bone (27). In agreement with this, in the present study no significant differences between both types of implants were found regarding primary stability. Periotest® values decreased over time without significant differences in the different time points.

In 2006 Noguerol et al. (28) analyzed 1084 Brånemark® implants inserted in 316 patients during a period of time of 10 years with the purpose of determining the accuracy of Periotest® compared with osseointegration analysis by x-rays. These authors concluded that Periotest® values higher than -2 for primary stability may indicate a poor and therefore risky osseointegration process. This is in agreement with the results of Periotest®, which reported that values higher than -2 indicate a good osseointegration for both implant groups.

In 2012 Payer et al. (29) analyzed primary stability of zirconia immediately loaded implants in 20 patients using Periotest®. They obtained negative values for all the periods of the study. The lowest values were recorded on the day of surgery (-1.89±1) and the highest values (-3.81±1.7) after 24 months, indicating a progressive decrease of Periotest® values.

The data of the present study also showed a decrease of Periotest® values in a 3-month period in both groups.

With respect to BIC, Dubruille et al. in 1999 (30) evaluated the quality of bone in contact to different implants in an experimental dog model without occlusal loading using zircona (Y-TZP), alumina and titanium implants. No significant differences were found between implants following macroscopic, microscopic and radiographic analyses. Cervical, central and apical BIC for each group of implants were also evaluated, obtaining higher values than in the present study for zirconia (Y-TZP) implants (47.94%). These results can be explained considering that the study period was shorter than in the present report. Moreover, no significant differences between titanium and Y-TZP implants were found regarding BIC and this is in agreement with studies by Dubruille et al. (30) and Stadlinger et al. (31), comparing osseointegration in zirconia Y-TZP vs titanium implants without occlusal loading in minipigs. After a healing period of 4 weeks, they did not find significant differences regarding BIC between both groups. In agreement with these authors, no significant differences between titanium and zirconia (Y-TZP) implants regarding BIC were also found in the present study in the first month of healing.

In 2008 Depprich et al. (12) concluded that zirconia (Y-TZP) implants with modified surfaces undergo an osseointegration comparable to titanium implants with the same modification surface. Their results at 1 month are similar to the values obtained in this study (44.68% for zirconia implants and 51.36% for titanium implants).

Finally, literature reviews (32, 33) concluded that BIC percentages of Yttrium partially stabilized zirconia implants (Y-TZP) are about 60%: similar values were obtained in the present study at 3 months (47.94%±16,15%) in zirconia implants modified by femtosecond laser (White-SKY®; Bredent medical® GMBH & Co. KG, Senden, Germany).

With respect to crestal bone loss, Albrektsson et al. (34) established that the marginal bone level change in the first year should be less than 1-1.5 mm and bone loss should be less than 0.2 mm in the following years. The results of the present study met Albrektsson success criteria with respect to crestal bone loss, because the highest crestal bone loss found in modified zirconia group at 3 months was 1.25 mm. In a study by Gahlert et al. (35), crestal bone loss was compared between standard SLA titanium implants and zirconia (Y-TZP) implants with fluoride acid etched surface. They found that 80% of titanium implants had a crestal bone loss of 3 or more threads after 4 weeks of healing, meanwhile 25% of zirconia (Y-TZP) implants with acid etched did not have any crestal bone loss sign at x-rays. These authors concluded that these differences in crestal bone loss may be due to the gap between implants that may favor septic microenvironment, or due to different wettability. For those reasons, they concluded that more studies are necessary to know the influence of these factors.

In conclusion, the present study demonstrated no differences for implant (primary and secondary) stability and BIC between zirconia and titanium implants, although, there was more crestal bone loss at Y-TZP group only at 3 months. Further investigations are necessary to analyze crestal bone loss at Y-TZP implants with this modification.

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