Histological and Histomorphometric Evaluation of Zirconia Dental Implants Modified by Femtosecond Laser versus Titanium Implants: An Experimental Study in Fox Hound Dogs

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ABSTRACT

Purpose: This study applied femtosecond laser technology to zirconia dental implants (Bredent GmbH & Co.KG, Senden, Germany) to generate a surface texture of microgrooves over the entire intraosseous surface, analyzing its behavior in an in vivo model in comparison with titanium implants with sandblasted and acid-etched surfaces.

Materials and Methods: The study used six American Fox Hound dogs. Each received four implants per hemi-mandible, making a total of eight implants per animal. The 48 immediate loaded implants were divided into two groups of 24 titanium implants (control group) and 24 zirconia implants (study/test group), distributed randomly. Bone-to-implant contact (BIC) values and crestal resorption were determined at 1 and 3 months, also measuring calcium, phosphorous, and carbon concentrations by means of energy dispersive x-ray.

Results: BIC percentages after 30 days were 51.36% for titanium implants and 44.68% for zirconia implants. After 90 days, values increased to 61.73% in titanium and 47.94% in zirconia implants. After 30 days, there was more crestal bone lost in the titanium group (0.77 mm) compared with the zirconia group (0.01 mm). After 90 days, zirconia implants showed greater marginal bone resorption (1.25 mm) compared with the titanium group (0.37 mm).

Conclusions: The present study shows that zirconia implants with modified surfaces can produce good osseointegration values when compared with titanium implants in terms of BIC and crestal bone resorption at 1 and 3 months.

KEY WORDS: BIC, crestal bone loss, zirconia implants

INTRODUCTION

In spite of the extensive worldwide use of titanium implants and their proven long-term reliability,[1–4] the literature describes some inconveniences arising from this choice of material, such as patient sensitivity to

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titanium and possible allergic reactions,[5–7] gingival retraction, or gingival translucidity in thin soft tissue biotypes, which can leave the dark grayish color of the titanium exposed to view in the upper maxilla with negative esthetic consequences.[8–10]

For these reasons, the use of zirconia dental implants has emerged in recent years as an alternative to titanium implants due to characteristics, which include excellent bone response, minimal inflammatory responses adjacent to their surfaces, biocompatibility, excellent optical and esthetic qualities, low bacteria and pathogen adherence, and high resistance to fracture and compression.[11–19]

The surface properties of zirconium dioxide (zirconia) make today impossible to produce the same textures as titanium implants by means of chemical and physical surface modifications. This is because zirconia is resistant to chemical treatment, especially acid attack, acid etching being a technique often used to treat titanium implant surfaces.[20]

The zirconia implants presently available on the market are subjected to surface sandblasting. The different physical microtexturing techniques that are currently applied to dental implants produce geometries and surface roughness values that are nanometrically and micrometrically random, of differing degree, and are not clean processes;[21] their reproducibility is arguable given that the outcomes of this type of processing are uncontrollable[22] and on occasions require special conditions, such as vacuums and the use of conductive materials. For this reason, the present study has adopted a promising technique, whereby precise control of texture can produce textures of complex shape, which can be achieved without the need for contact, does not cause contamination and is clean, and fast. This is microtexturing by laser,[23] which the study compares with sandblasted and acid-etched titanium, the gold-standard material for implant dentistry.

In recent years, high resistance ceramics have become attractive as new materials for dental implants as they are inert, undergo minimal ion release compared with metal implants, and possess excellent physical properties. Yttrium-partially stabilized zirconium dioxide (Yttria Tetragonal Zirconia Polycrystal [Y-TZP]) displays several advantages over aluminum oxide because of its high resistance to fraction and flexion,[13,24] as well as biocompatibility and low affinity to bacterial colonization.

The present study applied femtosecond laser technology to zirconia dental implant surfaces to produce a full microgrooved surface and analyzed its behavior and performance in an in vivo model, comparing the results with grade 4 cold-worked titanium implants with sandblasted and acid-etched surfaces.

MATERIALS AND METHODS

The study used six male American Fox Hound dogs, aged 1 to 3 years and weighing between 18 and 20 kg; the animals were provided by the University of Murcia (Spain), Research Support Service Animal Facility. The dogs all had intact mandibles, fully erupted permanent dentition, without any type of occlusal trauma and were in good general health. The animals were tagged for identification and fed and watered ad libitum.

The study protocol was approved by the Faculty of Medicine's Ethical Research Committee fulfilling regulations established by Royal Decree 1201 of October 10, 2005, Law 32 of November 7, 2007, and EU directive 2010/63/EU for the care of animals in transport, testing, and euthanization.

Study Design

The animals received 0.04 mg/kg acepromazine (Calmoneosan®, Pfizer, New York, NY, USA) as preanesthetic 10 minutes before receiving 0.2 mg/kg butorphanol (Torbugesic®, Fort Dodge Animal Health, Charles City, IA, USA) and 0.7 mg/kg medetomidine hydrochloride (Medetor®, Virbac, Burgdorf, Germany). The medication was injected intramuscularly in the femoral quadriceps. An infiltration injection of 1:100,000 articaine/epinephrine was administered in the surgical field to boost local anesthesia and reduce bleeding. These procedures were carried out under the supervision of a veterinary surgeon. Throughout the surgery, the animals' cardiac rate, blood oxygen saturation, and body temperature were monitored.

A crestal sulcular incision was made around the teeth raising a full thickness flap. The extractions of the premolars (P2, P3, P4) and first molar (M1) were performed in both hemi-mandibles of each dog. After extraction, the flaps were sutured using simple absorbable sutures (3-0 TB-15, Lorca Marin SA, Murcia, Spain).

After a 2-month healing period, implant insertion was performed. A total of 48 implants were divided into two groups; eight implants per animal were randomized by using the website http://randomization.com: control

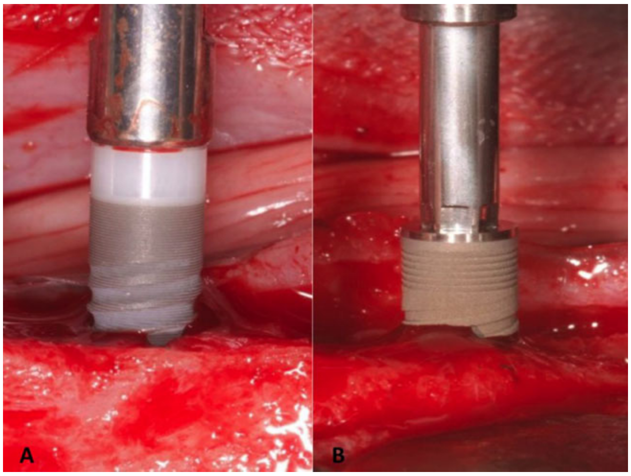

Figure 1 Implants placed. *A*, Zirconia implant. *B*, Titanium implant.

group: 24 titanium implants; test group: 24 zirconia implants (Bredent GmbH & Co.KG, Senden, Germany; Figure 1).

The 24 titanium implants were Blue SKY® (Bredent Medical® GmbH & Co. KG, Senden, Germany) of 4-mm diameter and 10-mm length, made from grade 4 cold-worked titanium, which maintains all mechanical properties, and received sandblasting to the surfaces, followed by acid etching.

The 24 zirconium dioxide implants, White SKY® one piece (Bredent Medical), were of 4 mm in diameter and 10 mm in length, modified by femtosecond laser over the entire intraosseous surface (Figure 2). This type of implant has the prosthetic post incorporated in the implant, and so, in order to fulfill the same load conditions as the control group, the post was cut off to leave the implant submerged after insertion. The surface texture modification was previously performed by the Laser Service at the Faculty of Physics of the University of Salamanca (Spain), following the technique described by Delgado-Ruiz and colleagues,[23] using a Tsunami® Ti:Sapphire oscillator (Spectra Physics, Newport Corporation, Alberta, Canada) that produces pulses of a hundred femtoseconds, near-infrared wavelengths (795 nm), and 10 nJ energy, with a repetition rate of 80 MHz.

Aveolar socket of 4-mm diameter and 10-mm length was prepared for each implant. Each hemi-mandible received four of the cylindrical implants, which all had the same dimensions and geometry in their intraosseous portion, inserted applying a torque of 35 Ncm or more in a submerged protocol. All the implants were immediate loaded by covers made from polyether ether ketone, were splinted using orthodontic ligature wire (0.16 mm), and reinforced with Pi-Ku-Plast HP 36 acrylic resin (Bredent Medical). The presence of occlusal contacts was manually checked with articulating paper with a thickness of 100 µm (Bausch Progress 100, Dr Jean Bausch KG, Kolhn, Germany) by inducing opening and closing movements. Suture was carried out using simple sutures to perform primary wound closure. The animals were sacrificed in two groups, 1 and 3 months following surgery, for analysis and clinical evaluation. The procedure by means of an overdose of pentothal natrium (Abbot Laboratories, Madrid, Spain) and perfused through the carotid

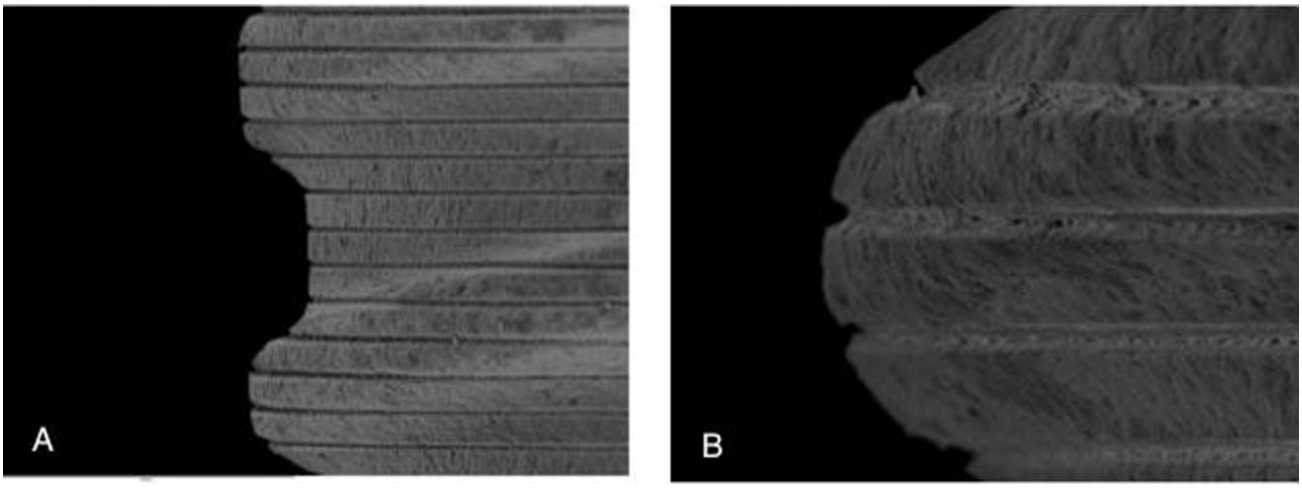

Figure 2 Detail of the implant used. *A*, Body implant (20×). *B*, Detail of the laser grooves (150×).

arteries with a fixative containing a mixture of 5% glutaraldehyde and 4% formaldehyde. The mandibles were dissected, and each implant site was removed using a diamond saw (Exakt Apparatebau, Norderstedt, Hamburg, Germany). Biopsies were processed for ground sectioning according to the methods described by Donath and Breuner.[25] Samples were dehydrated in increasing grades of ethanol up to 100%, infiltrated with metha-crylate, polymerized, and sectioned at the buccal-lingual plane using a diamond saw. Two sections were cut from each biopsy unit. The first was cut from the center of the implant and the second from the surrounding bone. Each block was sectioned with a high-precision diamond disk at about 100 mm thickness and ground to approximately 40 mm final thickness with a 400 s CS grinding device (Exakt Apparatebau).

Each section surface was stained using toluidine blue stain according to Schenk and colleagues,[26] and a semiquantitative evaluation of bone-to-implant contact (BIC) was made. To obtain a single digitally processible overview image of the whole zirconia and titanium implants per site, four images of the same implant were taken with a 10X objective and assembled into a single image. A 1-mm-wide zone around the implant surface reaching up to the original implantation level was defined as the region of interest (ROI). Within the ROI, the hard tissue was digitally defined into old bone and newly formed bone (Figure 3). In order to improve the differentiation between native and newly formed bone, light and dark blue chromaticity were enhanced by digital images. Finally, interface contact length between bone and implant surface (BIC) was determined.

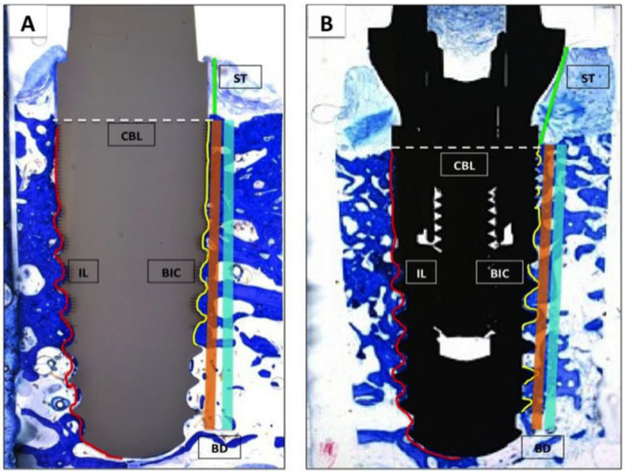

Figure 3 Parameters analyzed in this study. *A*, Zirconia implant; *B*, Titanium implant (CBL = crestal bone level; IL = implant length; ST = soft tissue).

BIC in each histological section was calculated by measuring the length of the implant surface in contact with bone tissue, in comparison with the total length of the implant surface, expressed as a percentage. To do this, the percentage of mineralized bone in direct contact with the titanium/zirconia surface was determined by counting inside the threaded zone. BIC percentages were calculated around the entire implant perimeter from the first point of BIC at the most coronal point, evaluating mineralized bone in contact with the implant surface linearly.[27] Histomorphometric analysis was performed using a video camera (Sony 3CCD, Sony, Berlin, Germany) with ×10 magnification. Images were digitalized (Axiophot-System, Zeiss, Oberkochen, Germany), stored, and reference points were plotted (crestal bone level [CBL]; implant length [IL]; soft tissue [ST]) (Figure 3).

The same images were also used for measuring crestal bone height. This was obtained by measuring the distance from the implant shoulder to the first point of BIC; measurements were taken for both titanium (control) and zirconia (test) implants at both study times.

A JEOL-6100 scanning electron microscope (Jeol Ltd, Tokyo, Japan) was used to evaluate BIC contacts and elemental analysis at the bone-to-implant interface. Elemental analysis was performed by means of energy dispersive x-ray (EDX) using an Oxford INCA 300 system (Oxford Instruments, Oxfordshire, UK). Analysis was performed between the second and third implant threads, inside the bone-to-implant interface.

Statistical Analysis

Analysis was performed using *SPSS* 15.0 statistical software (SPSS Inc., Chicago, IL, USA), licensed to the University of Murcia. Statistical significance was established as $p < .05$.

Means, medians, standard deviation, and standard error were calculated. A one-way ANOVA test was applied to compare two groups, and Post-Hoc testing and Bonferroni correction for comparing averages between groups.

RESULTS

Histomorphometric Findings

Tables 1 and 2 show the results of histomorphometric measurements. BIC evaluation produced a mean of 51.36 ± 12.03% for titanium (control) implants after

TABLE 1 Bone-to-Implant Contact Values at 1 and 3 Months of Titanium and Zirconia Implants

	Titanium	Zirconia	*p*
1 month	51.36 ± 12.03%	44.68 ± 17.66%	1
3 months	61.73 ± 16.27%	47.94 ± 16.15%	0.678
p	0.09	0.749	

The level of statistical significance was set at $p < .05$.

1 month of healing and of 61.73 ± 16.27% after 3 months ($p = .09$); femtosecond laser-modified zirconia (test) implants showed mean BIC of 44.68 ± 17.66% after 1 month and 47.94 ± 16.15% after 3 months ($p = .74$), with no significant differences between study periods. Table 1 gives a comparison of BIC values between titanium and zirconia implants.

Crestal Bone Resorption

As shown in Table 2, crestal bone resorption was higher in the titanium implants during the first month following surgery (Group I) (0.77 ± 0.69 mm) than after 3 months (0.37 ± 0.34 mm). For zirconia implants, crestal bone resorption was greater after 3 months (1.25 ± 1.73 mm) than after the first month (0.01 ± 0.57 mm), but the standard deviation obtained at the 3-month mark was similar to the mean value.

It is of note that the lowest crestal bone resorption values were obtained by zirconia after 1 month (0.01 ± 0.57 mm). However, zirconia also showed the highest bone loss at the 3-month evaluation (1.25 ± 1.73 mm).

The data obtained show that for zirconia implants, there was no significant difference between study periods (at 1 and 3 months), whereas for titanium implants, there was a significant difference. Zirconia showed better behavior after 1 month than titanium, but this was reversed at the 3-month study time when titanium showed better behavior.

Elemental Analysis

Comparing carbon (C) percentages at 1 and 3 months in both groups, for titanium implants, C decreased from 15.77 to 11.68%, respectively. However, for zirconia, the opposite occurred, with C increasing from 12.53 to 13.48%, respectively (Table 3).

The highest phosphorous (P) percentage was found in the titanium group at 3 months (4.25%), which had increased from 3.76% after the first month. In the zirconia group, the P percentage was 3.85% after a month and 3.91% after 3 months.

As for calcium, the highest percentage was found in the titanium implant group at 3 months (17.01%); the lowest was seen in the zirconia group (15.97%) at 3 months (Table 3).

DISCUSSION

The present research was based on an earlier work by Delgado-Ruiz and colleagues[23] who modified the surface of zirconia implants using femtosecond laser to generate a grooved texture. There is evidence in the literature that these microgrooves allow better movement of the cells over the surface. Using an in vitro model, Delgado-Ruiz and colleagues demonstrated how surfaces treated with femtosecond laser to create 30-µm wide grooves guide and stimulate cell growth and increase osteoblast adhesion inside the grooves.

Calvo-Guirado and colleagues[28] founded that zirconia femtosecond laser all treated surface showed better results subjected to immediate loading versus those that remained unloaded. Comparing BIC values, immediate loading achieved a higher percentage. The histomorphometric results obtained at 30 and 90 days after implant placement show a statistically significant improvement in BIC in the immediately loaded group compared with the conventional nonloaded group, and peri-implant crestal bone resorption was less at 30 and 90 days for immediately loaded all treated laser zirconia implants compared with nontretated zirconia implants. The authors' literature review did not find any studies analyzing BIC values for zirconia implants subjected to immediate or delayed loading. This may be because of the novelty of zirconia in implant dentistry. In this way, the first study was pioneering research with regard to this issue.

TABLE 2 Values Crestal Bone Resorption in the Titanium and Zirconia Implants Group at 1 and 3 Months

	Titanium	Zirconia	*p*
1 month	0.77 ± 0.69 mm	0.01 ± 0.57 mm	0.07
3 months	0.37 ± 0.34 mm	1.25 ± 1.73 mm	0.03*
p	0.024*	0.133	

*The level of statistical significance was set at $p < .05$.

TABLE 3 Measuring Different Analyzed Elements over the Surface of the Implants

	1 Month		3 Months	
	Titanium	Zirconia	Titanium	Zirconia
Ca	16.25 ± 0.44%	15.97 ± 0.88%	17.01 ± 0.45%	16.35 ± 1.09%
P	3.76 ± 0.19%	3.85 ± 0.45%	4.25 ± 0.37%*	3.91 ± 0.3%
				$p < .05$
C	15.77 ± 0.38%*	12.53 ± 0.53%	11.68 ± 0.68%	13.48 ± 0.8%*
		$p < .05$		$p < .05$
Ca/P Ratio	4.32 ± 0.03	4.15 ± 0.04	4 ± 0.03	4.18 ± 0.06

*The level of statistical significance was set at $p < .05$. Ca, carbon; P, phosphorous.

Following on from this research, the present study at 1 and 3 months used an in vivo experimental model to analyze the behavior of full laser-modified zirconia implants and compared these with titanium implants, the material most commonly used in implant dentistry. In BIC evaluations at the study times, both titanium and femtosecond laser-modified zirconia implants osseointegrated similarly without significant differences for this variable. Therefore, it can be confirmed that the introduction of microgrooves on the entire intraosseous zirconia surface will produce equivalent clinical outcomes to titanium implant surface treatments (sandblasting and acid etched).

The present results are similar to those obtained by Stadlinger and colleagues[29] in a study comparing osseointegration of zirconia implants with titanium implants inserted in a minipig model, without occlusal functional loading. Zirconia implants were placed using two healing protocols (submerged and nonsubmerged), whereas titanium implants were all placed in submerged position. After a 4-week healing period, BIC was analyzed demonstrating no significant differences in osseointegration. Therefore, the BIC percentages between the two implant types was 53% for titanium implants, 53% for nonsubmerged zirconia implants, and 48% for submerged implants. Likewise, the present study did not show significant differences between titanium and zirconia implants. By comparing these values with those obtained in the present assay, it can be seen that marginal bone resorption may be influenced by other parameters such as implant design or material, rather than the condition of subjection to immediate or delayed loading. In this way, Romanos and colleagues concluded that, in addition to the benefits that the use of immediate loading can offer the patient, long-term results are favorable even in areas where bone quality is inadequate.[30]

Depprich and colleagues[16] also studied osseointegration of partially stabilized zirconia implants (Y-TZP) inserted in minipigs, comparing them with titanium implants after treating both implant types with acid etching. BIC was analyzed in the intraosseous portion at 1, 4, and 12 weeks. At 4 weeks, a BIC percentage of 45.3 ± 15.7% was found for zirconia implants and 47.7 ± 9.1% for titanium implants; at 12 weeks, the BIC was 71.4 ± 17.8% for zirconia and 82.9 ± 10.7% for titanium; no significant differences were identified between the implant types, although the higher BIC values found might be explained by the fact that implants were placed in an aseptic environment – the tibia – whereas in the present study, the implants were inserted in the mouth. Depprich and colleagues concluded that zirconia implants with modified surfaces could obtain osseointegration similar to that achieved by titanium with the same surface modification.

Andreiotelli and colleagues[31] and Wenz and colleagues,[32] in systematic literature reviews, concluded that BIC percentages achieved by Y-TZP are around 60% in the literature published to date, a value that concurs with the results of the present study.

As for crestal bone resorption values, it is noteworthy that the lowest values were obtained by zirconia implants at the 1-month study time (0.01 ± 0.57 mm). However, the zirconia implants also showed the highest crestal bone loss at the third month (1.25 ± 1.73 mm), showing how zirconia implants suffer less resorption in earlier healing stages, although not significantly. Nevertheless, titanium implants were seen to suffer less crestal bone loss in the longer term.

Cannizzaro and colleagues[33] investigated whether immediate, nonocclusal, one-piece provisionalization of partially stabilized zirconia (Y-TZP) implants could reduce early failure rates in comparison with immediate occlusal loading protocols. The study monitored 40 patients divided into two groups, placing provisional prostheses on the same day as implant surgery, either with or without occlusal contact. After 4 to 5 months, the definitive prostheses were placed. Among the success criteria assessed in the study, peri-implant marginal bone levels were measured by x-ray using the paralleling technique. After a year of occlusal loading, the patients not subjected to immediate loading had suffered a mean peri-implant bone loss of 0.7 mm, in comparison with 0.9-mm bone loss among patients subjected to immediate loading after 1 year; however, the difference was not statistically significant.

Nickenig and colleagues[34] analyzed two titanium implant collar designs, one with a polished collar and the other with microgrooves. The authors performed a radiographic follow-up at four study times: on the day of surgery, after osseointegration, at 6 months following functional loading, and at 2 years following surgery, obtaining significantly lower bone loss values in the group of microgrooved implants (0.1 mm during the osseointegration period, 0.4 mm at 6 months, and 0.5 mm after 2 years), in comparison with the implants with polished collars. Similar values were obtained in the present study for the titanium implants (sandblasted and acid etched), 0.77 mm after 1 month, and 0.37 mm after 3 months.

The rate of trabecular bone remodeling in dogs is around 100% per year, whereas in humans, it is between 5 and 15% per year.[35] When this difference is taken into account, it can be seen that the results of the present study (whereby there was an average crestal bone loss of 1.25 mm after 3 months for zirconia implants) are comparable with the data obtained by Nickenig and colleagues.[34]

EDX microanalysis identified slightly more Ca in the control group, showing significant differences in P concentrations at 3 months and in C ratios at 1 and 3 months. Ca/P ratio values had been previously analyzed in dogs and did not differ from the values found.

Ca and phosphorous values increased for both implant materials as the study progressed, a fact that is related to higher rates of bone regeneration in the bone neoformation process that takes place during osseointegration. The results were similar for both groups, which indicate that both materials behave similarly during bone healing.

In conclusion, the present study shows that zirconia femtosecond laser all treated surface presents good osseointegration values when compared with titanium implants in terms of BIC and crestal bone resorption after 1 and 3 months. This means it can be a valid option and safe in the implantological daily practice.

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