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A scoping review on the surface modification of zirconia dental implants by laser texturing

Welson Figueiredo da Cunha

Dissertação conducente ao Grau de Mestre em

Medicina Dentária (Ciclo Integrado)

Gandra, 30 de junho de 2020

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Trabalho realizado sob a Orientação de "Júlio César Matias de Souza"

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O Orientador

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"Tenha em mente que tudo que você aprende na escola é trabalho de muitas gerações (...) Receba essa herança, honre-a, acrescente a ela e, um dia, fielmente, deposite-a nas mãos de seus filhos."

Albert Einstein

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RESUMO

O objetivo deste trabalho foi realizar uma revisão integrativa da literatura sobre a influência da irradiação a laser para aprimorar os aspetos topográficos da superfície de implantes de zircônia e o processo de cicatrização óssea. Foi realizada uma pesquisa eletrônica na base de dados PUBMED, usando os seguintes termos de pesquisa: "zirconia" AND "laser" AND "surface modification" OR "surface treatment" AND "dental implants" OR "bone" OR "osteoblast" OR "osseointegration". Dos artigos identificados, 15 estudos foram selecionados nesta revisão.

Os resultados relataram que a irradiação com laser foi capaz de promover alterações morfológicas nas superfícies de zircônia com aumento da rugosidade e molhabilidade. O aumento na rugosidade foi registrado em escalas micro- e manométrica e resultou em um aumento na molhabilidade e adsorção de proteínas. Além disso, a adesão, a disseminação, a proliferação e a diferenciação de células osteogênicas aumentaram após a irradiação a laser, principalmente com um laser em fentosegundo, 10nJ de energia, e frequência de 80 MHz. Após 3 meses de osseointegração, os estudos *in vivo* em cães revelaram uma percentagem média de contato osso-implante para superfícies de zircônia (em torno de 47,9 ± 16%) quando comparados às superfícies padrão de titânio (61,73 ± 16,27%), demonstrando não haver diferença significativa entre os diferentes materiais.

A técnica de irradiação a laser revelou vários parâmetros que podem ser usados para modificar a superfície da zircônia, como intensidade, tempo e frequência. Os parâmetros do laser podem ser ainda otimizados e controlados para alcançar uma modificação da superfície e resposta biológica desejáveis tendo em consideração ao processo de osseointegração.

Palavras-chave: Zircônia; tratamento da superfície; laser; implantes dentários; osseointegração

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ABSTRACT

The aim of this work was to perform an integrative literature review on the influence of laser irradiation on zirconia implants to enhance surface topographic aspects and the biological response for bone healing. An electronic search was carried out on the PUBMED database using the following search terms: "zirconia" AND "laser" AND "surface modification" OR "surface treatment" AND "dental implants" OR "bone" OR "osteoblast" OR "osseointegration". Of identified articles, 15 studies were selected in this review.

Results reported that the laser irradiation was capable to producing changes on the zirconia surfaces regarding topographic aspects, roughness, and wettability. An increase in roughness was recorded at micro- and nanoscale and it resulted in an increased wettability and biological enhancement. Also, adhesion, spreading, proliferation, and differentiation of osteogenic cells were enhanced after laser irradiation mainly by using Femtosecond laser at 10nJ and 80MHz. After 3 months osseointegration, *in vivo* studies in dogs revealed an average percentage of bone-implant contact for zirconia surfaces (around 47.9 ± 16%) when compared to standard titanium surfaces (61.73 ± 16.27%), demonstrating that there is no significant difference between the different materials.

The laser irradiation technique revealed several parameters that can be used for zirconia surface modification such as intensity, time, and frequency. Laser parameters can be optimized and well-controlled to reach desirable surface morphologic aspects and biological response concerning the osseointegration process.

Key words: Zirconia; surface treatment; laser; dental implants; osseointegration

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1 - INTRODUCTION

In implant dentistry, osseointegration has been studied considering the direct, structural, and functional connection between bone tissue and implant surfaces over occlusal loading. (1–5) The long term stability of dental implants depends on the chemical composition and surface of implants materials although the health state of patients also affect osseointegration. (6–8) On standard titanium dental implants, several physicochemical techniques have been successfully used for enhanced osseointegration such as double acidic etching and grit-blasting. (9–11) However, the surface modification of zirconia surfaces revealed different outcomes taking into consideration ordinary physicochemical techniques. (11–17) At first, the surface topographic aspects are quite different when compared to those noticed on titanium surfaces. (18) Second, the ordinary acidic etching has no effect on zirconia surfaces regarding roughness. (16,18) In this way, advanced surface modification methods have been developed to modify zirconia surfaces and maintaining the physical performance of the material. (18–24)

Zirconia is a chemically stable and biocompatible ceramic used for implants and prosthetics in orthopedics and dentistry. (10,25,26) The chemical stability of zirconia becomes a challenge concerning the surface modification. (16,18) The physical properties of zirconia are achieved by stabilizing the tetragonal zirconia phase at room temperature by incorporating small contents of oxides: Y_2O_3, MgO, CeO_2, or CaO. (25,26) For instance, yttria stabilized tetragonal zirconia polycrystals (YTZP) has a flexural strength at 900 - 1200 MPa, elastic modulus of approximately 210 GPa , and fracture toughness at around 7-10 MPa.m$^{1/2}$. (26) *In vitro* and *in vivo* studies have demonstrated the osseointegration capability of Y-TZP zirconia implants quite similar to those on titanium implants since the surface modification techniques are well applied. (11–17) Grit-blasting is the major physical method to increase the titanium or zirconia roughness for adsorption of proteins, attachment of osteogenic cells, and bone formation. (6,13,14,27,28) Micro- and nano-scale surface modification influences the adsorption of extracellular matrix proteins, which regulate the adhesion of osteoblasts to the implant surface leading to cell proliferation and differentiation. (6,11,13,28) However, modification of zirconia cannot be controlled by using only ordinary surface modification such as grit-blasting.

Various methods of surface treatment have been proposed to improve the surface properties of zirconia implants. (16,27,29–31) Currently, surface modification on the implant surface via laser irradiation has gathering attention regarding the increase in roughness, wettabillity, and biological response without affecting the physical properties of zirconia. (19,21–23) Morphological features (e.g. micro-grooves, pits, valleys, and peaks) on material surfaces can be controlled by using different intensity, type, time, and frequency of laser irradiation. (7,32) The fentosecond laser allows the reuse of sulcus and pores on the surface of zirconia implants, and can be used with different power intensity settings, (19,21,33,34) with a power of 30mW was able to produce grooves with a depth of 25μm and width of 30μm. (19) The CO2 laser, depending on the power intensity used, is capable of producing different patterns of surface microstructure. (22,23) Other types of lasers (e.g. Nd:YAG, ErCr:YSGG and fiber) are also capable of altering the surface of the zirconia producing different results in the surface quality of the treatments. (24,35,36)

The main aim of this study was a literature review about the biological response to laser surface treatment in zirconia implants. It was hypothesized that laser treatment is able to improve the surface characteristics of zirconia implants by promoting a more effective biological response.

2 - MATERIALS AND METHOD

An electronic search was performed on the PUBMED database using the following search items: "zirconia" AND "laser" AND "surface modification" OR "surface treatment" AND "dental implants" OR "bone" OR "osteoblast" OR "osseointegration". The inclusion criteria involved articles published in English language, up to May 20th, 2020 reporting studies on the modification of zirconia surfaces by laser irradiation. The eligibility inclusion criteria used for article searches also involved: articles written in English; meta-analyses; randomized controlled trials; and prospective cohort studies. The total of articles was gathered for each combination of key terms and therefore the duplicates were deleted using Mendeley citation manager. Two of the authors (JCMS, WFC) independently evaluated the titles and abstracts of potentially relevant articles. A preliminary evaluation of the abstracts was carried out to establish whether the articles met the purpose of the study. Selected articles were individually read and evaluated concerning the purpose of this study. The following factors were retrieved for this review: author names, journal, publication year, purpose, zirconia types, roughness, biological response, bone-to-implant contact (BIC) percentage, and laser parameters such as intensity, exposure time, laser type, wavelength, application mode.

3 - RESULTS

The bibliographic search on PUBMED identified a total of 101 articles, as shown in Fig. 1. After excluding duplicates, 46 articles were evaluated by title and abstract although 31 were excluded because they did not meet the inclusion criteria. The remnant 15 articles were full read and then considered relevant to the purpose of the present study. Thus, 15 studies were included in this review.

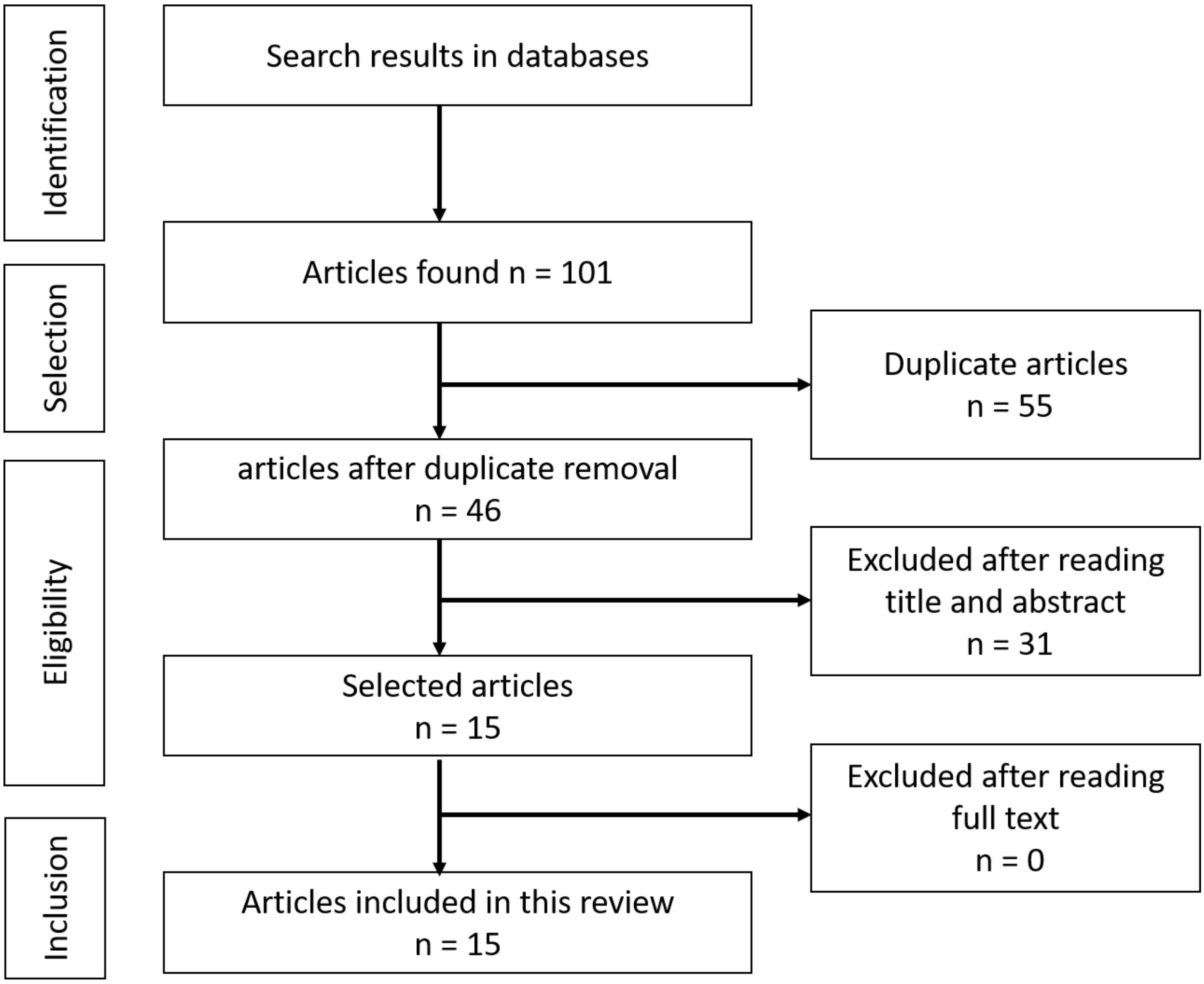

Figure 1: Study selection flowchart

Of the 15 selected studies, 7 (46.7%) were carried out *in vitro* while 8 (53.3%) were performed *in vivo*. The bone to implant contact (BIC) was evaluated by 7 studies (46,7%) while 3 (20.0%) studies evaluated the resorption of the bone crest. Laser surface treatment was morphologically characterized by 10 (66.7%) in vitro articles and 6 (40.0 %) evaluated the cellular response to the laser surface treatment. The main outcomes can be drawn as follow:

- The laser treatment of the surface promoted changes in the topography of the implant surface at following the levels of scale: mesoscale (grooves), micro-scale (peak/valley), nanoscale (nodules). (37) The crystalline structure of the zirconia was not altered after laser-treatment leading to the maintenance of the tetragonal phase and decreasing the residual monoclinic phase. (19,21) A nanoscale rough surface was noted in the micro-grooves of zirconia surfaces. (21,35,38,39)

- The laser surface treatment was favorable to the adhesion, proliferation and differentiation of osteoblasts on the zirconia surfaces, with increase of 1 to 8 times of cell proliferation in the laser-treated groups compared to control groups. (22,23,36)

- In animal models, the proportion of bone-implant contact (BIC) on laser-treated zirconia implants showed mean values that allow them to be considered as a valid option in the clinical practice of implantology. (20,34,40,41) When compared to the laser treatment produced on the surface of YTZP zirconia implants with the treatment by blasting and HF, the treatment produced by the laser provided a higher degree of BIC. (35) However, laser-treated zirconia showed similar BIC values to the titanium surfaces treated by ordinary grit-blasting and etching procedures. (20,41)

- Also, the laser-treated implants showed good levels of peri-implant bone crest maintenance in the animal models. (20,33,40) Early loaded implant promoted a less bone crest loss (0.5 ±0.23 mm) in comparison with late loaded implants (0.56 ± 0.28mm) over a period of 90 days. (40)

Table 1: Relevant data extracted from the selected studies

Authors (YEAR)	Study Design	Sample size	Follow Up	Laser parameters	Analyses	Main outcomes
Aivazi *et al.*, (2016)(19)	*In vitro*	A-3Y-TZP20 Nano-composite discs, with an average grain size of ≤ 400nm.	ND	Femtosecond laser (Legend Elite Coherent Inc. company, USA), with polarized pulses at 800 nm at 1000 Hz and 30mW, 0,50mm/s	• SEM - Surface characterization, Element analysis; • XRD - Chemical and Phase composition; • FESEM -structural analysis	• The surface of the A-Y-TZP20 samples was modified to the micro-groove pattern. Reduction of contaminants incorporated in the previous stages of manufacture. • The material around the microstructures did not show phase transformation.
Calvo-Guirado *et al.*, (2013)(20)	Animal study • 6 male American Foxhound dogs aged 1 to 3 years and weighing between 18 and 20 kg. Each dog received 8 implants.	48 implants, two groups: • control - titanium implants. • test – zirconia Y-TZP implants White SKY® (Bredent Medical)	• 1 month • 3 months	Tsunami® Ti: Sapphire oscillator (Spectra Physics, Newport Corporation, Alberta, Canada) that produces pulses of a hundred femtoseconds, near-infrared wavelengths (795nm), and 10nJ energy, with a repetition rate of 80MHz.	• SEM – BIC; • EDX - Elemental analysis; • Histomorphometric analysis and measuring crestal bone height.	1 month: • BIC Ti implants - 51.36% • BIC zirconia implants - 44.68% Marginal bone resorption: • Zirconia implants (0,01mm); • Titanium implants (0,77mm). 3 months: • BIC Ti implants - 61.73% • BIC zirconia implants - 47.94% Marginal bone resorption: • Zirconia implants (1.25mm); • Titanium implants (0.37mm).

Calvo-Guirado *et al.,* (2014)(40)	Animal study • 6 male American Foxhound dogs aged between 1 and 3 years and weighing from 18 to 20 kg. Each dog received 8 implants.	48 zirconia implants White Sky® (4mm x 10mm) • 24 with immediate loading; • 24 without loading	• 30 days • 90 days	Femtosecond laser	• SEM, OM – BIC; • Periotest – Stability; • X ray - crestal bone height.	After 30 days • BIC - immediately loaded group – 38.9% and non-loaded 32%. • Crestal bone resorption in the non-loaded group (0.58 ± 0.28mm), immediately loaded group (0.5 ± 0.3mm). After 90 days • BIC - immediately loaded group - 65% and 57.6% for non-loaded. • Crestal bone resorption in the immediately loaded group (0.5 ± 0.23mm), non-loaded group (0.56 ± 0.28mm).
Calvo-Guirado *et al.,* (2014)(34)	Animal study • 20 male New Zealand rabbits aged 30–35 weeks and weighing 3900–4500g, placing two implants per tibia	80 implants, four groups: • Group A: titanium implants, sandblasted and acid-etched • Group B: zirconia implants and sandblasted • Group C: titanium implants, sandblasted and acid-etched, supplemented with MLT 5% in solution. • Group D: zirconia implants, sandblasted and micro grooved by femtosecond laser, supplemented with MLT 5% in solution	• 1 week • 4 weeks	Femtosecond laser Ti: Sapphire oscillator (Tsunami; Spectra Physics, Santa Clara, CA, USA) The system delivers 120fs linearly polarized pulses at 795nm with a repetition rate of 1kHz. Pulse energy can reach a maximum of 1.1mJ	• SEM, OM and EDX - BIC.	At 1 week: • Group C (29.7 ± 2.4%) and group D (28.9 ± 1.3%) implants showed higher BIC % compared with group A and B. After 4 weeks: • Group D showed higher BIC compared with all the groups (47.5 ± 2.2%).

Delgado-Ruiz *et al.,* (2011)(21)	*In vitro*	66 cylindrical zirconia implants (3Y-TZP) 4mm x 8mm, three groups: • control group (without laser modification), • Group A (implants treated with femtosecond laser pulses to create pores), • Group B (implants treated with femtosecond laser pulses to create grooves).	ND	Femtosecond laser Ti: Sapphire oscillator (Tsunami, Spectra Physics). The system delivers 120fs linearly polarized pulses at 795nm with a repetition rate of 1kHz.	• Optical interferometric profilometry - surface roughness; • SEM - Surface characterization; • X-ray diffraction - change in crystalline structure; • EDX - Elemental analysis.	• Ultra-fast laser ablation increased the surface roughness (Ra, Rq, Rz and Rt) significantly for the two texture patterns, from 1.2x to 6x times when compared to the control group ($p <0.005$). • Significant decrease in contaminants such as carbon (Control 19.7% ± 0.8%> Group B 8.4% ± 0.42%> Group A 1.6% ± 0.35%) and aluminum (Control 4.3% ± 0.9%> Group B 2.3% ± 0.3%> Group A 1.16% ± 0.2%) on laser treated surfaces ($p <0.005$). • The tetragonal phase was preserved, while the traces of the monoclinic phase present on the treated surfaces were reduced (Control 4.32%> Group A 1.94%> Group B 1.72%).
Delgado-Ruiz *et al.,* (2014)(33)	Animal study • 12 Foxhound dogs of approximatel y one year of age, each weighting between 14 to 15 kg, each mandible received 8 implants	96 implants (4 mm x 10 mm), four groups: • control group - Ti implants • group A -sandblasted zirconia implants; • group B –zirconia implants treated with femtosecond laser pulses over 2 mm from the neck area; • group C –zirconia implants treated with femtosecond laser pulses over the entire intraosseous surface	• 1 month • 2 months • 3 months	Femtosecond laser	• SEM • EDX - Elemental chemical composition analysis; • Perioteste – Stability; • X ray - crestal bone height.	• Periotest values increased in all periods, proportional to the extent of the microgrooves, as follows: Group C > control > group B > group A ($p <0.05$). • Minimal bone loss at 3 months for implants in groups C, B and control compared to implants in group A ($p <0.05$). • The implant surfaces of groups B and C showed extra bone growth inside the micro grooves that corresponded to the shape and direction of the microgrooves.

Hao *et al.*, (2004)(23)	*In vitro* Human osteoblastic cell line hFOB 1.19, • 1x10^5 cell/mL (cell fixation and morphology: 24h), • 4x10^5 cells/mL (cell growth analysis: 7 days) • 1x10^5 cell/mL (cell count analysis: 14 days)	Blocks with 50 x 12 x 2.15 mm of zirconia partially stabilized with 4% magnesia (MgO - PSZ)	• 24 h • 7 days • 14 days	CO_2 laser: 3kW, used at: • 0.6kW/cm^2, • 0.9kW/cm^2, • 1.6kW/cm^2, • 1.9kW/cm^2, • 2.5kW/cm^2, 10.6μm wavelength, travel speed was adjusted to 2000 mm/min.	• SEM - Cell culture and adhesion; • Sessile drop measuring machine (First Ten Ångstroms, Inc) – Wettability; • Profilometer - surface roughness; • SEM, OM, XRD - microstructure and crystal size.	Cell growth, compared with the untreated one: • (0.6 kW/cm^2), increase 17%; • (0.9 kW/cm^2), double cover density; • (1.6 kW/cm^2), triple cover density; • (1.9 kW/cm^2), triple cover density; • But the cell coverage area did not increase further at higher power density of 2.5. Surface roughness (Ra): • 0.295 μm (untreated), • 0.305 μm (0.6kW/cm^2), • 0.333 μm (0.9kW/cm^2), • 0.717 μm (1.6kW/cm^2), • 1.882 μm (1.9kW/cm^2), • 3.854 μm (2.5kW/cm^2). Wettability: Glicerol • 79° (untreated), • 76° (0.6kW/cm^2), • 62° (0.9kW/cm^2), • 40° (1.6kW/cm^2), • 50° (1.9kW/cm^2), • 54° (2.5kW/cm^2).

Hao *et al.,* (2004)(39)	*In vitro.* • Acellular human SBF (ion concentration almost equal to that of human blood plasma)	Blocks with 50x12x2.15 mm of zirconia partially stabilized with 4% magnesia (MgO – PSZ)	• 14 days.	CO_2 laser: 3kW, used at: • 0.6kW/cm², • 0.9kW/cm², • 1.6kW/cm², • 1.9kW/cm², • 2.5kW/cm², 10.6μm wavelength, travel speed was adjusted to 2000 mm/min.	• Sessile drop measuring machine (First Ten Ångstroms, Inc) – Wettability; • Profilometer - surface roughness; • SEM, OM, XRD - microstructure and crystal size.	The Ra increased as the laser power density increased. The surface roughness (Ra) was: • 0.295μm (untreated), • 0.305μm (0,6kW/cm²), • 0.333μm (0,9kW/cm²), • 0.717μm (1X6kW/cm²), • 1.882μm (1,9kW/cm²), • 3.854μm (2,5kW/cm²). The shape of the surface microstructure varied with the different power densities of the CO_2 laser applied: • Crystal rearrangement (0.6kW/cm²), hexagonal structure (0.9kW/cm²), cell formation (1.6kW/cm²) uniform cell formation (1.9kW/cm²) and coral and dendritic (2.5kW/cm²). • Improve the bioactivity of the MgO-PSZ surface, generating a functional group to facilitate the formation of bone apatite's.
Hao *et al.,* (2005) (22)	*In vitro* • Human osteoblastic cell line hFOB 1.19, 4×10⁵ cells/mL	5% yttria partially stabilized zirconia (YPSZ)	• 1 week	CO_2 laser: 3kW, used at • 1.80 kW/cm², • 2.25 kW/cm². 10.6μm wavelength, travel speed was adjusted to 5000mm/min	• SEM - Cell culture and adhesion; • Sessile drop measuring machine (First Ten Ångstroms, Inc) - Wettability	• In the treated samples, there was a decrease in surface roughness and a solidified microstructure. Increased wettability characteristics and better adhesion of osteoblastic cells. Wettability: Glicerol • 82.4° (untreated), • 74.2° (1.80kW/cm²), • 70.5° (2.25kW/cm²), The shape of the surface microstructure varied with the different power densities of the CO_2 laser applied: hexagonal structure (1.8kW/cm²), cell microstructure (2.25kW/cm²).

Hirota *et al.*, (2019)(35)	Animal study • 12 male Wistar rats, each weighing approximately 180g and 6 weeks old. Each animal received an implant, in the femur.	12 implants (rectangular plates 3x2x1mm) • G1- laser/3Y-TZP; • G2- laser/A-10Ce-TZP30; • G3 – blasted HF/3Y-TZP; • G4- blasted HF/A-10Ce-TZP30.	• 4 weeks	Nd:YAG nanosecond-pulsed laser 3 ns; 150μJ/pulse; 1,064nm; 50Hz; 7μm/s.	• SEM - Surface characterization; • EDX - Elemental analysis; • CLSM –BIC and bone formation.	• Production of nanoscale surface topography inside the micro grooves of Y-TZP and Ce-TZP. • Laser treatment was effective to increase BIC for • Y-TZP (78.9%±6.57%), but not for Ce-TZP (14.0%±2.43%). BIC of laser implants/Y-TZP was significantly the highest among the four different implants ($p < 0.05$). • Surface chemistry influenced bone formation separately from surface morphology.
Hoffmann *et al.*, (2012)(41)	Animal study • 48 female New Zealand White rabbits weighing between 2.0 and 2.5 kg each. One implant was placed in each distal rear femur of each rabbit, with a total of two per rabbit	96 implants (3.2x6mm) four groups: • (G1) - 24 zirconia with a sintered surface; • (G2) – 24 zirconia with a laser-modified surface; • (G3) – 24 zirconia with a sandblasted surface (control 1); • (G4) – 24 titanium with an acid-etched surface (control 2)	• 6 weeks; • 12 weeks	ND	• OM - BIC	BIC 6 weeks: • sintered zirconia - 32.996%±14.192%; • laser-modified zirconia - 39.965%±13.194%; • sandblasted zirconia - 39.614%±15.029%; • titanium implants - 34.155%±15.816%. BIC 12 weeks: • sintered zirconia - 33.746%±14.529%; • laser-modified zirconia - 43.87%±14.544%; • sandblasted zirconia - 41.350%±15.816%; • titanium implants - 34.818%±12.209%.

Nassif *et al.*, (2018)(38)	*In vitro* • Cell line used was Saos-2 (primary human osteosarcoma) $50x10^3$/mL	80 zirconia disks (19.5x3mm): $ZrO_2 + HfO_2 + Y2O_3$: >99.0 Y_2O_3: 4.5–5.6 HfO_2: < $5Al_2O_3$: < 0.5; Other oxides: < 0.5) Four groups: • Group I: sintered (AS - control); • Group II: Rocatec (ROC); • Group III: Laser (LAS). • Group IV: SIE.	• 3 days • 1 week	Er, Cr:YSGG laser (BIOLASE, California, USA) frequency - 20Hz; power - 3W	• VHN - Microhardness; • AFM - Surface morphology and topography; • SEM - Microstructural • Components; • Hemocytometer – Cell number and density	Cell count: • 3 days – SIE (53.5 ± 2.2) > AS (51 ± 1.4) > LAS (23 ± 1.9) > ROC (21.5 ± 1). • 7 days - SIE (108 ± 1.7) > AS (72 ± 2.1) > LAS (37.5 ± 1.2) > ROC (32 ± 1.4) Surface Roughness (Ra): • ROC (2.201µm ±0.352µm) > LAS (1.412µm ±0.166µm) > SIE (0.830µm ± 0.098µm) > AS (0.475µm±0.027µm).
Soares *et al.*, (2016)(24)	*In vitro* MC3T3-E1 osteoblast cells, line derived from mouse tissue, $1x10^4$ cells/mL	48 Y-TZP blocks (92% ZrO_2, 5% $Y2O_3$, HfO_2<3%, $Al2O_3$, SiO_2 <1%) were divided into 4 groups: • G1 (no laser irradiation); • G2 (1.5W); • G3 (3.0W); G4 (5.0W).	• 3 days • 7 days	Laser Er,Cr:YSGG las (Waterlase, Biolase Technology Inc, Irvine, CA; wavelength of 2780nm). Used at: • 1.5W; • 3.0W; • 5.0W; 30 seconds; 20Hz	• Confocal White Light Microscope - surface roughness topography; • SEM - zirconia surface morphology, cellular morphology; • MTT - cell adhesion and proliferation	Surface Roughness (Ra): • G1 (1.26±0.6), • G2 (1.52±2.0), • G3 (1.14±0.7), • G4 (0.70±0.3). After 3 days, cell response higher than the control group: • G2 = 1,4%, • G3 = 3.1%. • G4 = 4,5% After 7 days, there was no difference between groups.

Rezaei *et al.*, (2018)(37)	*In vitro* • Osteoblasts derived from the bone marrow of the femur of Sprague - Dawley rats, 3×10^4 cells/cm^2 Animal study • 12 male Sprague-Dawley rats, eight weeks old (femur)	Zirconia (Y-TZP) in disc (20mm in diameter, 1.5mm in thickness) and cylindrical shape (1mm in diameter, 2mm in length). Two groups: • Y-TZP with machined surface, • laser engraved rough surface.	• 6 h • 24 h • 3 days • 5 days • 7 days • 14 days • 2 weeks • 4 weeks	ND	• SEM - Surface morphology; • ESCA - Chemical composition of the surface; • XPS - Chemical composition of the surface; • WST-1 - Cell density; • qPCR - Gene expression; • EDX - Elemental analysis; • OM - Surface morphology; • LM - osteoblast morphology and scattering; • Push-in test - strength of bone-implant integration.	Surface morphology: • The rough surface of the Y-TZP was characterized by grooves on a meso scale (50µm wide, 6–8µm deep), microscale valleys (1–10µm wide, 0.1–3µm deep) and nanoscale nodules (10 -400 nm wide and 10-300 nm high), while the machined surface was flat and uniform. The average roughness (Ra): • Of the laser-treated Y-TZP was five times greater than that of machined zirconia. Cell density: • The number of osteoblasts adhered to the zirconia surfaces after 6 and 24 hours of culture was equivalent between the machined and laser treated surfaces. Push-in test in the two and four weeks: • 2.2-times greater for hierarchical rough zirconia implants than machined zirconia implants.

Taniguchi *et al.*, (2015)(36)	*In vitro* • MC3T3-E1 $1.4x10^4$ cells/cm^2 Animal study • 16 male Sprague-Dawley rats, 8 weeks old. Each rat received S-ZrI in one tibia and R-ZrI in the other tibia	*In vitro:* • 132 zirconia plates (Y-TZP; 10x10x1mm). Two groups: • smooth polished surface (66 S-Zr), • Rough surface created with a fiber laser (66 R-Zr). Animal study: 32 implants (1,6x8mm). Two groups: • Smooth polished surface (16 S-ZrI), • Rough surface created with a fiber laser (16 R-ZrI).	• 6 h • 24 h • 3 days • 7 days • 14 days • 4 weeks	Fiber laser (MD-F3000, Keyence, Osaka, Japan)	• Laser microscope - surface roughness; • SEM - surface roughness, cell morphology; • OM - cell morphology; BIC; • WST - cell proliferation; • PCR - osteoblastic activity.	Cell proliferation: • was significantly greater in R-Zr than in S-Zr, in 3 days. • R-Zr index was 1.2 times higher on days 7 and 14 days. Surface roughness: • The values of surface roughness indexes Sa of R-Zr were 9.7 times greater than that of S-Zr, and the Sdr value of R-Zr was 7.8 times greater than that of S-Zr. • In the implant, the Sa value of R-ZrI was 13.0 times greater than that of S-ZrI, and the Sdr value of R-ZrI was 37.8 times greater than that of S-ZrI. BIC: • The BIC ratio for the cortical bone side was 2.1 times higher for R-ZrI than for S-ZrI, while the ratio for the bone marrow side was almost the same for R-ZrI and S-ZrI.

4 – DISCUSSION

4.1 - Zirconia implants

Zirconia has revealed remarkable clinical outcomes in the biomedical field, mainly in orthopedics and dentistry, due to the biological and response. (10,14,25,26,42,43) In the last years, zirconia has been used to develop teeth root canal posts, orthodontic brackets, and implant abutments, and prosthetic infrastructures. (9,44,45) Since 2004, the zirconia implants commercially available regarding aesthetic, mechanical, and biological peri-implant outcomes. (5,13,17,45–51)

Zirconium dioxide (ZrO_2) known as zirconia is a polymorphic ceramic, which has three distinct crystallographic phases: monoclinic (m), tetragonal (t) and cubic (c). (26,52,53) At room temperature pure ZrO_2 has a monoclinic structure that remains stable up to 1170 ° C. (26) It turns to the tetragonal zirconia when sintering in the temperature between 1170 and 2370 °C while the cubic phase is reached between 2370 and 2680 °C. (26) Zirconia as a ceramic biomaterial On cooling, the ZrO_2 tetragonal phase becomes monoclinic at a temperature around 970 °C. (26) The transformation pathway of tetragonal to the monoclinic phase is associated with approximately 3 to 4% volumetric expansion that can lead to cracks. (26,52) The mechanical properties of the zirconia are enhanced when the tetragonal phase is stabilized by adding small content of oxides such as yttrium oxide or yttria (Y_2O_3), magnesium oxide (MgO), cerium oxide (CeO_2), and calcium oxide (CaO). (26,52–55) For instance, yttria-stabilized polycrystals zirconia (YTZP) is produced by adding 2-5 mol% yttria in the zirconia. (56) That results in a significant increase in the flexural strength values at around 1200 MPa, elastic modulus at 230-270 GPa, and fracture toughness of approximately 9- 10 MPa. $m^{1/2}$. (17,45,52,53,57) On high stresses, oxide-stabilized zirconia has an inherent mechanism to inhibit the propagation of cracks. That consists in a transformation of the tetragonal to the monoclinic phase with an increase in the surrounding volume leading to the compression of the crack. (26) However, zirconia is susceptible to degradation at low temperature when used in a humid environment as found in the oral cavity. (26) Recurring tetragonal-to-monoclinic phase transformation can result in fatigue by cyclic stress from mastication loading and thermal oscillations. In this way, additive oxides such as alumina, ceria, or silica has been used to improve the degradation resistance of zirconia. (25,26,58)

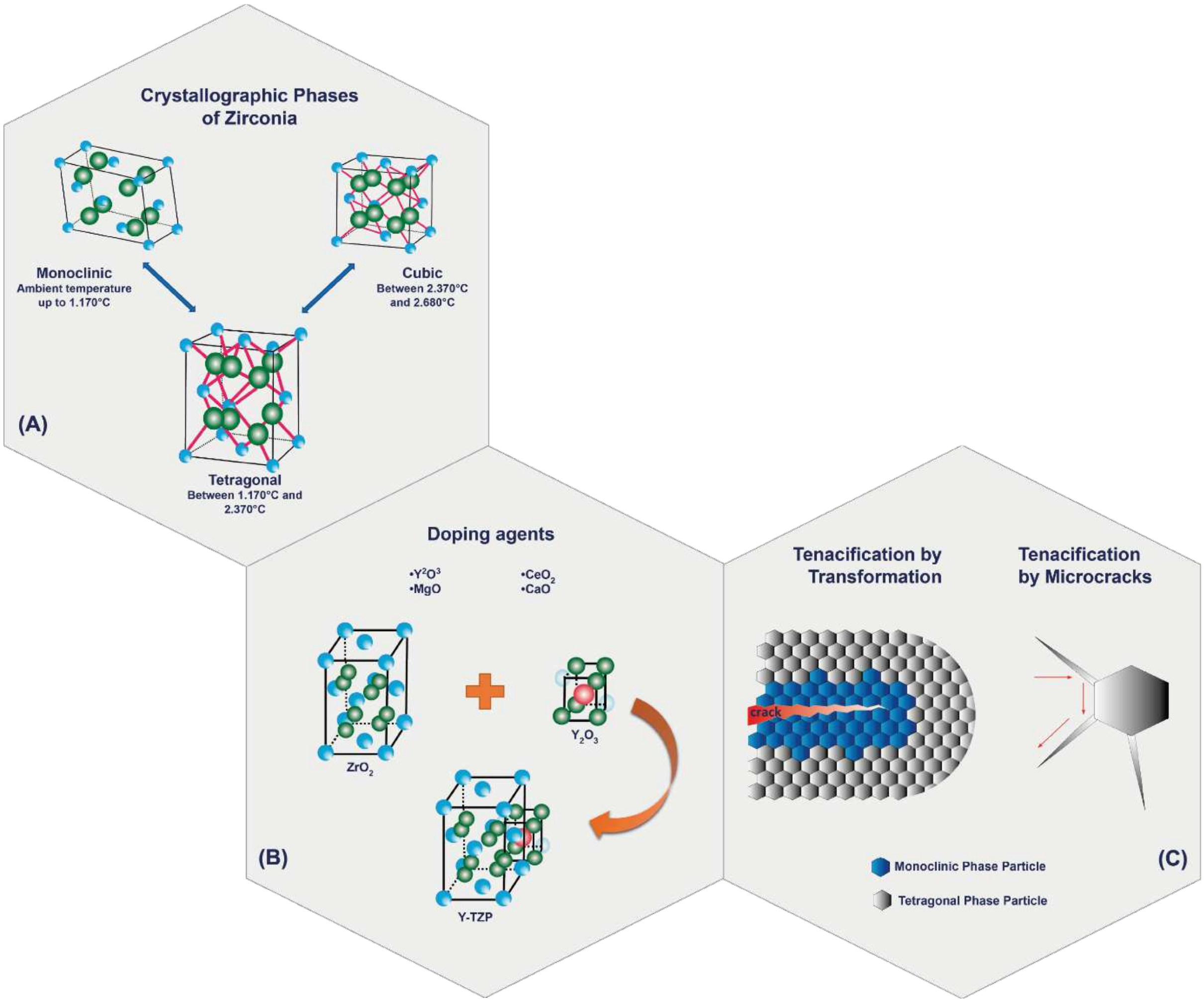

Figure 2: A) Crystalographic phases of zirconia. B) Doping agents. C) Zirconia tenacification mechanism.

Regarding surface, topography, roughness, and chemical composition control the wettability and adsorption of proteins and ions (e.g. Ca^{+2}, PO^{-}, OH^{-}) onto the zirconia prior to the osseointegration. (22,23,39) Then, the activation of blood platelets and osteogenic cell migration follow the formation of the primary bioactive layer composed of ions and proteins. (7,46,59–61) The differentiation of osteogenic cells and further formation of collagen matrix and bone tissue depend on the surface features and chemical interaction. (7,36) Several surface modifications have been proposed to enhance the surface such as grit-blasting, calcium-based coatings, ultraviolet irradiation, and laser-structuring protocols. (15,20,24,27–31,62) Nevertheless, zirconia surface modification is a current challenge considering a balance among physical properties, chemical stability, and degradation behavior. (63) Nowadays, most zirconia implants commercially available undergo surface treatment by grit-blasting, which produce non-homogeneous and random surface features under high risks of degradation. (11) The crystalline structure of the zirconia was not altered after laser-treatment leading to the maintenance of the tetragonal phase and decreasing the residual monoclinic phase. (19,21) Surface modification of zirconia by laser-structuring has been studied and therefore different procedures can be applied regarding the laser type, intensity, time, and mode. (19–24,36) In fact, the wettability and roughness of zirconia surfaces can be enhanced by laser-structuring maintain the degradation resistance, biocompatibility, and chemical interaction with the surrounding medium. (21–23,36,39)

4.2 - Zirconia surface modification

Zirconia implants are often machined by CAD-CAM that results in surfaces with grooves or scratches leading to an average roughness (*Ra* parameter) at around 0.2-0.4 µm. (22,36,37) Similar roughness values (~0.3 µm) were reported on machined MgO-PSZ. (23,39) Wettability of the MgO-PSZ was measured by the contact angle of glycerol droplet of around 79° (23) while machined YPSZ revealed a mean value of 82.4°. (22) The average roughness of sintered YTZP was measured at around 1.2 ± 0.6 µm. (24) Sintered YTZP implants are produced with rough surfaces once the peak and valleys depend on the zirconia powder particle size. (19) On the standard surfaces of YTZP implants modified by the grit-blasting method, the average roughness of machined YTZP can be increased due the abrasive effect of airborne particles. (21,33) As a consequence, morphological features such as peaks and valleys are randomly distributed over the grit-blasted and sintered zirconia surfaces. The average roughness of YTZP grit-blasted surfaces has been measured ranging from 1.2 to 1.6 µm. (21,33)

The morphological aspects of zirconia surfaces can be controlled by using the laser irradiation approach. Well-designed grooves, scratches, valleys, and peaks are produced at macro- and micro-scale width (1-100 µm) and micro-/submicron-scale depth (0.1-10 µm). (37) The roughness and wettability of laser-treated surfaces can also be adjusted considering the implant region, as seen in Table 1. (21,23,33,39) In a previous study, a femtosecond laser irradiation was used to produce micropore patterns with 30 µm diameter and 70 µm pitch and micro-grooves with 30 µm width and 70 µm pitch on Y-TZP. The average roughness values of the micropores' patterns reached 2.4 ± 0.6 µm while micro-grooved surfaces showed roughness values of 9.5 ± 0.6 µm. That resulted in an effective surface contact area of 15% for micro-porous surfaces and 25% for micro-grooved surfaces. (21,33) Femtosecond laser irradiation was also applied on A-Y-TZP surfaces leading to regular micro-grooves' patterns with 30 µm width and 25 µm depth. (19) Granular polycrystalline structures with dimensions of 1-6 µm were detected, where nanostructures with sizes ranging from 30 to 100 nm were observed. (21) In fact, the modification at micro and nano-scale increased the surface contact area for interaction with proteins and osteogenic cells.

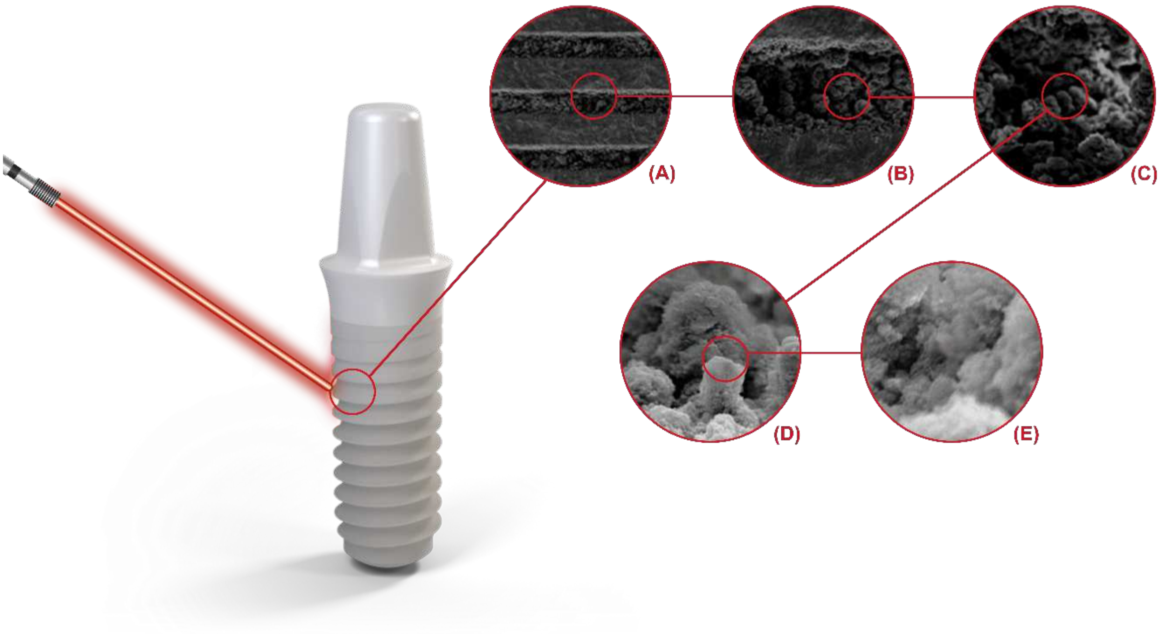

Figure 3: Representation of the laser grooved surface of a progresssive enlarged zirconia implant.

Previous studies reported the modification of MgO-PSZ surfaces by using CO_2 laser regarding different laser intensity. (23,39) Morphologic aspects of the surfaces varied in function of the intensity such as: crystal refurbishment on 0.6 kW / cm^2; hexagonal structure on 0.9 kW / cm^2); pores formation (1.6 kW / cm^2); and dendrite (2.5 kW / cm^2). (23,39) Consequently, *Ra* roughness increased as the power density of the laser irradiation was augmented (Table 1): Ra of 0.3 μm on 0.6 kW / cm^2; 0.33 μm on 0.9 kW / cm^2; 0.71 μm on 1.6 kW / cm^2); 1.8 μm on 1.9 kW / cm^2; and 3.8 μm on 2.5kW / cm^2. (23,39) On the MgO-PSZ surfaces, the angle of contact of the glycerol droplet decreased as the roughness increased that indicates an increase in wettability: 76° on 0.6 kW / cm^2); 62° on 0.9 kW / cm^2; 40° on 1.6 kW / cm^2; 50° on 1. 9 kW / cm^2; and 54° on 2.5 kW / cm^2. (23) However, a significant decrease in the *Ra* roughness was detected on YPSZ when the CO_2 laser intensity was increased although the morphological aspects also varied as a hexagonal microstructure appeared on 1.8 kW / cm^2, while a porous microstructure was noted on 2.25 kW / cm^2. The values of the contact angle of the glycerol droplet also decreased with the increase in laser power: 74.2° on 1.80 kW / cm^2 was found while an angle of 70.5° was found on 2.25 kW / cm^2. (22)

On Er, Cr: YSGG laser at low level irradiation, no significant changes were detected on YTZP using the following parameters: 1.5 W (Ra of 1.52 ± 2.0 µm and Sa of 1.78 ± 2.0 µm); 3.0 W (Ra of 1.14 ± 0.7 µm and Sa of 1.24 ± 1.3 µm); and 5.0 W (Ra of 0.70 ± 0.3 µm and Sa of 1.36 ± 1.0 µm). (24) However, micro-scratches and shallow grooves were detected in another study assessing a laser irradiation of 3 W on YTZP surface that resulted in a *Ra* roughness at 1.41 ± 0.166 µm and *Rz* roughness at 5.1 ± 0.327 µm. (38) A fiber laser was also used to produce changes on the YTZP surfaces and therefore the results revealed regular edges' pattern leading to an increase in roughness at around 10 times (Sa of 1.75 ± 0.32 µm) when compared to machined surfaces (Sa of 0.18 ± 0.04 µm). (36)

4.3 - Biological response to surface characteristics produced by laser

In vitro studies on cell culture have shown a stimuli of the osteogenic cell response on laser-treated zirconia, as illustrated in Table 1. (22–24) For instance, zirconia surface treated with CO_2 revealed a significant an increase in the osteogenic cell proliferation by 70-90% when compared to untreated zirconia. (22,23) The laser energy used in the surface treatment of zirconia has an active effect on the osteogenic cell behavior. (22–24) The osteogenic cell proliferation can be increased at when the laser intensity was increased. (22–24) A progressive increase in cell proliferation was noted on Y-TZP irradiated with Er, Cr: YSGG laser with wavelength of 2780 nm, for 30 seconds and 20 Hz at 1.5 W, 3 W and 5 W when compared to untreated zirconia. (24) However, another study with Er,Cr:YSGG laser at 20 Hz and 3 W reported a significantly smaller difference in cell proliferation for 3 and 7 days cell incubation. Viable cell count was measured at (23 ±1.9 x 10^3) and (37.5 ± 1.2x10^3) for laser-treated Y-TZP and (51 ±1.4 x 10^3) and (72 ±2.1x10^3) for untreated zirconia. (38)

The morphological aspects of the osteoblast have been reported in previous studies by evaluating projections of cytoplasm, named phillopodia, and the spreading of the cell over the surfaces. (22–24,36,37) The cells exhibited a final stage of cell adhesion, showing more flattened and with a higher cytoplasmic projection with phyllopod that extended about 50-60 µm beyond the cells when compared to smaller philopods (5 to 10 µm projections) on untreated surfaces. (22,23) The degree of maturation achieved by osteoblasts after contact with leaser treated YTZP surfaces is another important aspect to be considered, since it is a key factor for the production of the bone matrix. (36,37) A higher degree of cell differentiation on laser-treated surfaces was validated by measuring osteogenic genes, such as collagen type I, osteopontin, osteocalcin, and BMP-2. (37) Results showed values ranging from 7 up to 25 times higher for laser-treated Y-TZP compared to untreated surfaces over a period of 7-days incubation. (37) Remarkable changes in cell morphologic aspects was evaluated by Taniguchi *et al* (36) at approximately 3 times for irradiated YTZP when compared to the non-irradiated Y-TZP. (36) The morphologic changes were linked to an increase in the gene expression of Runx2 mRNA, alkaline phosphatase, and oxytocin mRNA for 3, 7, and 14 days incubation, respectively. (36) Those are essential transcriptional factors for the differentiation of osteoblasts. (64) Thus, laser-based surface modifications increased the gene expression related to time-dependent osteogenic differentiation.

Studies have shown similar BIC values for titanium or zirconia implant surfaces treated with laser irradiation. (20,34,41) In dogs American Foxhound model, mean bone to implant contact (BIC) percentage was recorded at 44.6 ± 17.6% for zirconia for 1 month and 47.9 ±16 % for 3 months. No statistically differences were found when compared to titanium implant surfaces regarding the BIC mean values at 51.3 ±12 % for 1 month and at 61.7 ±16.2% for 3 months. (20) In another study in dogs American Foxhound model, laser-treated zirconia implant surfaces showed the BIC mean values at 22.8 ± 1.5% for 1 week and 37.5 ± 2.1% for 4 weeks. (34) Those values were also not statistically different when compared to BIC values for titanium surfaces: 25.4 ±1.2% for 1 week and 38.4 ± 1.8% for 4 weeks. (34) Similar results were found in another study in New Zealand white rabbits regarding the BIC mean values recorded on laser-treated zirconia implants at 39.97 ± 13.19% for 6 weeks and 43.87 ± 14.54% for 12 weeks in comparison to BIC mean values on titanium implant surfaces at 34.15 ± 10.34% for 6 weeks and 34.82 ± 12.21% for 12 weeks. (41) Thus, laser treatment is able to modify the topography of the zirconia surface, improving osseointegration and generating BIC values similar to the surfaces of titanium implants. (20,34) BIC studies in Wistar rats showed no statistically difference between YTZP (56.2 ± 3.56%) and CeTZP (37.1 ± 14.01%) with sandblasted surface treatment and acid attack for four weeks. (35) However, the laser-treated YTZP surfaces by using Nd:YAG wavelength 104 nm, pulse of 3ns, 50 Hz and 150 mJ/pulse revealed higher BIC mean values (78.9 ± 6.57%) when compared to laser-treated CeTZP (14.0 ± 2.43%). (35)

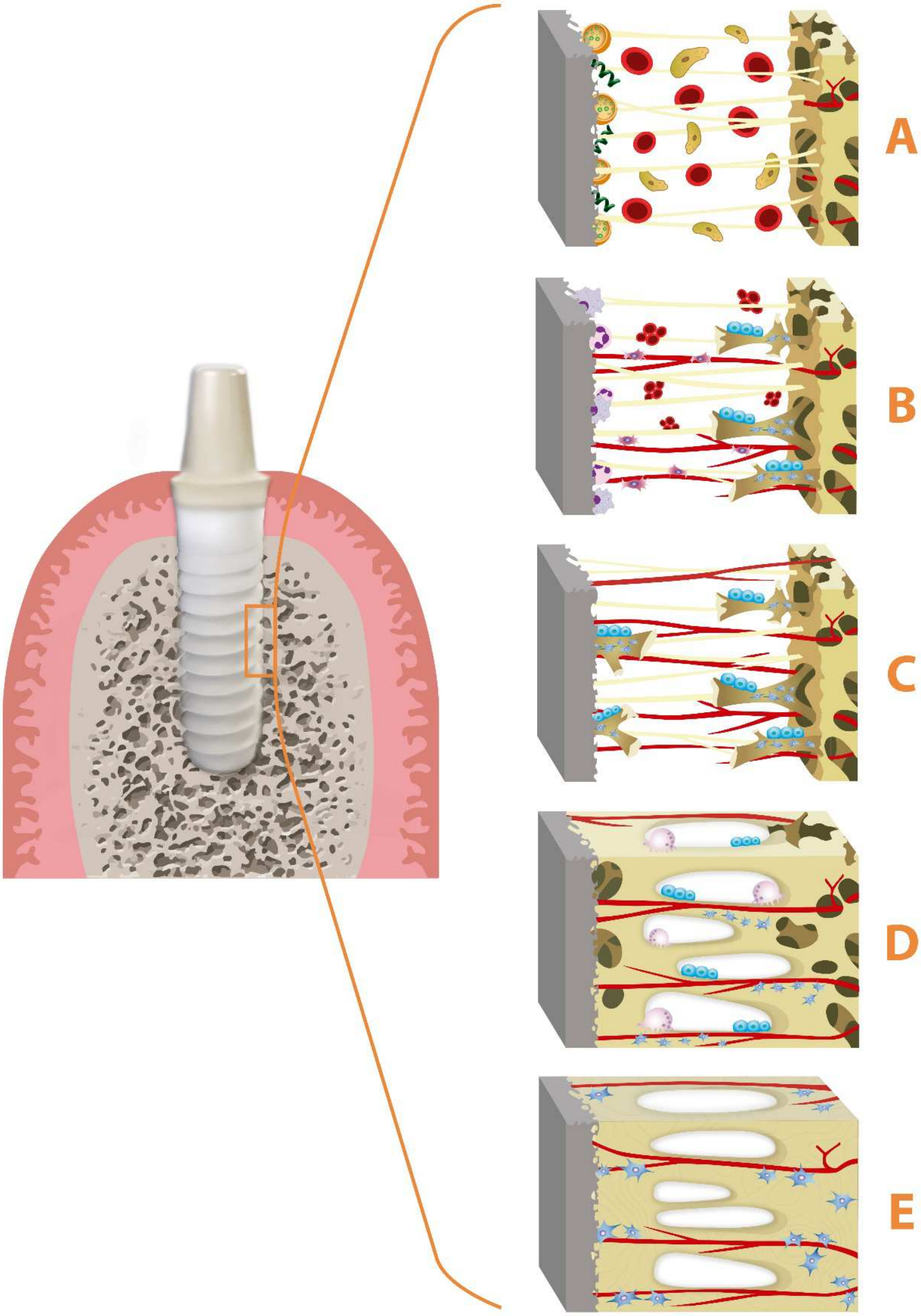

Figure 4: Scheme of the osseointegration process. A) Formation of the blood clot and fibrin matrix. Protein adsorption to the implant surface from the blood clot. B) Angiogenesis and formation of bone tissue. C) Distance osteogenesis and contact osteogenesis. D) Newly formed bone tissue. E) Mature bone tissue.

In the comparison between machined and laser-treated YTZP implants, in the period of four weeks, the BIC mean values was 2 times higher (81.9 ±20.4%) in the cortical bone portion in the Sprague-Dawley rats hen compared to those for machined YTZP surfaces (39.8 ± 19.2%). In the cancellous bone portion, BIC mean values did not show significant differences. (36) In the evaluation of the BIC percentage between zirconia implants with different surface treatments, no statistically significant differences were found between the YTZP surfaces with different surface treatments for 6 or 12 weeks respectively: 33 ±14% and 33.7 ±14.5% on sintered zirconia; 39.6 ±15 % and 41.3 ±15.8% on gritblasted; and 39.97 ± 13.19% and 43.87 ± 14.54% on laser treated surface. (41) Regarding occlusal loading, Y-TZP implants with Femtosecond, subjected to immediate loading, showed higher BIC values for 1 month (38.9 ± 6.68%) and 3 months (65 ± 4.36%) when compared to the same implant condition free of occlusal loading (32 ± 3.65% for 1 month) and (57.6 ± 3.62% for 3 months). Findings revealed a statistically significant improvement of the BIC percentage when implants were immediately loaded. (40)

In a Foxhound dogs model, Y-TZP zirconia implants treated with femtosecond laser, near-infrared wavelengths 795 nm and 10 nJ energy with a 80 MHz, showed marginal bone crest resorption values of at 0.01 ± 0.57mm for 1 month and at 1.25 ± 1.73mm for 3 months. (20) These values were statistically significant only in the three-month period when compared to the marginal bone crest resorption values in titanium implants: 0.77 ± 0.69 mm for 1 month and 0.37 ± 0.34 mm for 3 months. (20) Another study reported findings on Y-TZP zirconia implants treated with femtosecond on the entire implant body or only on the implant neck. (33) After 3 months, zirconia implants treated with laser at the neck region revealed a higher crestal bone loss (0.36 ± 0.01 mm) when compared to the implants treated in the entire contact surfaces (0.26 ± 0.01 mm). (33) Immediately loaded Y-TZP zirconia implants showed crestal bone loss values of 0.5 ± 0.3 mm for 1 month and 0.5 ± 0.23 mm for 3 months. (40)

5 – CONCLUSIONS

1. The surface treatment using the laser generated changes in the surface roughness parameters, producing microstructures in meso, micro and nanoscale, in addition to promoting better surface wettability. It is a clean and safe method that, due to the superficial changes produced, improves the biocompatibility of zirconia.

2. The laser treatment produced a favorable response in the initial levels of adhesion and dissemination of osteoblasts on the zirconia surface, besides promoting proliferation and differentiation of these cells.

3. Laser treatment is able to preserve bone crest levels and promote greater bone growth in addition to increasing the BIC on the zirconia surface. Similar values of bone-implant contact were found in titanium implants and laser-treated zirconia implants.

Perspectives: Further study is needed to establish ideal power parameters for each type of laser used in the surface treatment of the most varied zirconia ceramics found on the market, with the objective of producing optimal effects of surface roughness and energy, aiming at a better biological response.

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