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PÉRSIO VASCONCELOS MIRANDA

ESTUDO DAS ALTERAÇÕES SUPERFICIAIS DE UM SUBSTRATO CERÂMICO E TITÂNIO RUGOSO IRRADIADO COM LASER DE Er,Cr:YSGG

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Guarulhos, 28 de Fevereiro de 2012.

Dedico esta dissertação ao meu Pai,
Pérsio Miranda.

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“Você pode ficar desapontado se falhar, mas já estará derrotado se não tentar.”

Beverly Sills

“A confiança em si mesmo é o primeiro segredo do êxito.”

Ives Vaiet

RESUMO

Objetivos: O objetivo deste estudo foi investigar os efeitos do laser de érbio cromo dopado com ítrio, escândio, gálio e granada (Er,Cr:YSGG) com parâmetro de descontaminação, na rugosidade superficial (Sa) em zircônia tetragonal policristalina estabilizada com ítrio (Y-TZP) e titânio com jateamento de partículas grandes e ataque ácido (SLA) (TI). **Métodos:** Quinze discos de Y-TZP (AS Technology Titanium FIX, São José dos Campos, Brasil) com 5 mm de diâmetro e 3 mm altura padronizados com CAD-CAM (computer-aided design e computer-aided manufacturing) foram usados. Dez discos de 5 mm de diâmetro e 4 mm de espessura de titânio (SLA) (AS Technology Titanium FIX, São José dos Campos, Brasil) foram usados. Os discos de Y-TZP foram distribuídos em três grupos para irradiação com o laser (n=5): Y-TZP_G1= controle (sem irradiação); Y-TZP_G2= Y-TZP irradiado com laser Er,Cr:YSGG (1,5 W; proporção resfriamento ar-água 80%/25%); Y-TZP_G3= Y-TZP irradiado com laser Er,Cr:YSGG (1,5 W; proporção resfriamento ar-água 80%/0%). Os discos de titânio SLA foram distribuídos em dois grupos para irradiação com laser (n=5): TI_G1= titânio SLA controle (sem laser); TI_G2= titânio SLA irradiado com laser Er,Cr:YSGG (1,5 W; proporção resfriamento ar-água 80%/25%). A superfície do material foi analisada por um microscópio confocal de luz branca. A média dos valores de Sa (μm^2) e o desvio padrão foram calculados por meio de 5 perfis de cada grupo. Os valores foram analisados estatisticamente por *one-way* ANOVA com 95% de nível de confiança e comparados pelo teste de Tukey (α=0,05) para cada material. **Resultados:** ANOVA mostrou diferença estatisticamente significativa para o fator “laser” (p=0,00179) para Y-TZP. Os resultados (μm^2) foram: Y-TZP_G1: 2,589 (1,098)A; Y-TZP_G2: 0,815 (0,171)B; Y-TZP_G3: 0,746 (0,391)B. ANOVA mostrou diferença estatisticamente significativo para o fator “laser” (p= 0,00949) para o titânio SLA. Os resultados (μm^2) foram: TI_G1: 1,990 (0,507)B; TI_G2: 3,367 (0,754)A. **Conclusões:** Er,Cr:YSGG utilizado no parâmetro de descontaminação alterou a rugosidade da superfície da Y-TZP e do titânio SLA.

PALAVRAS-CHAVES

zircônia; discos de titânio; microscopia confocal; implante dentário

ABSTRACT

Objectives: The aim of the present study was investigate the effects of erbium chromium-doped: yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser irradiation with decontamination parameter on surface roughness (Sa) for yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) and sandblasted large grit acid-etched (SLA) titanium (TI) material. **Methods:** Fifteen disks of Y-TZP (AS Technology Titanium FIX, São José dos Campos, Brazil) with 5 mm diameter and 3 mm high standardized with CAD-CAM (computer-aided design and computer-aided manufacturing) were used. Ten disks of 5 mm in diameter and 4 mm in thickness of titanium (SLA) surface (AS Technology Titanium FIX, São José dos Campos, Brazil) were used. The Y-TZP disks were randomized in three groups to laser irradiation (n=5): Y-TZP_G1= control (no laser treatment); Y-TZP_G2= Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/25%); Y-TZP_G3= Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/0%). The titanium disks were randomized in two groups to laser irradiation (n=5): TI_G1= Titanium SLA control (no laser treatment); TI_G2= titanium SLA irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/25%). The material surface was analyzed by confocal white light microscopy. The mean of Sa values (μm^2) and standard deviation were calculated from five profiles of each group. Values were statistically analyzed by one-way ANOVA at the 95% confidence level and compared by Tukey Hoc post-test (α=0.05) for each material. **Results:** ANOVA showed statistical significant differences for the factor “laser” (p=0.00179) for Y-TZP material. The results (μm^2) were: Y-TZP_G1: 2.589 (1.098)A; Y-TZP_G2: 0.815 (0.171)B; Y-TZP_G3: 0.746 (0.391)B. ANOVA showed statistical significant differences for the factor “laser” (p= 0.00949) for titanium SLA material. The results (μm^2) were: TI_G1: 1.990 (0.507)B; TI_G2: 3.367 (0.754)A. **Conclusions:** Er,Cr:YSGG used on parameter of decontamination alters the roughness surface of zirconia Y-TZP and titanium SLA.

KEY WORDS

zirconia; titanium disks; confocal microscopy confocal; dental implants

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

A reabilitação com implantes de titânio para pacientes parcial ou totalmente desdentados tem altos índices de sucesso (BUSER et al., 1997; AL-NAWAS et al., 2012). Muitos estudos mostram a influência das características da superfície de implantes de titânio como um fator relevante para o sucesso da osseointegração (SHIBLI et al., 2007; SCHWARZ et al., 2009). Em contraste com superfícies usinadas e lisas, as superfícies micro ou nano estruturadas mostram um aumento da interface osso-implante (BIC) favorecendo a osseointegração (ROMANOS et al., 2006; D'ÁVILA et al., 2010).

Recentes avanços no desenvolvimento de materiais com alta resistência mecânica tornaram a zircônia uma alternativa para a confecção de implantes odontológicos (DELGADO-RUIZ et al., 2011). O arranjo espacial dos átomos da zircônia apresenta uma propriedade conhecida como polimorfismo, que ocorre em três formas distintas a sua estrutura cristalográfica: monoclínica, cúbica e tetragonal (DIAS JR, 2011; KOHAL et al., 2008; DENRY & KELLY, 2008). As zircônias podem ser classificadas em três tipos de acordo com a sua microestrutura: FSZ (*fully stabilized zirconia*), PSZ (*partially stabilized*) e TZP (*tetragonal zirconia policristals*) (CHEVALIER et al., 2009). A adição de uma pequena concentração de estabilizadores à zircônia pura mantém a sua estrutura na fase tetragonal em temperaturas superiores a 1.000°C e a uma mistura de fases cúbica e monoclínica ou fase tetragonal em temperaturas menores. A zircônia estabilizada é também conhecida como zircônia tetragonal policristalina (DIAS JR, 2011; DENRY & KELLY, 2008) e óxido de zircônia estabilizado com ítrio (Y-TZP, zircônia policristalina tetragonal estabilizada com ítrio) é biocompatível (DELGADO-RUÍZ et al., 2011; STUBINGER et al., 2008; SUBASI & INAN, 2011).

Pilares transmucosos em zircônia (van BRAKEL et al., 2011; BRESSAN et al., 2011) assim como implantes totalmente em zircônia são atualmente disponíveis no mercado (GAHLERT et al., 2010; STUBINGER et al., 2008). As suas características estéticas e mecânicas superiores a outras cerâmicas (SUBASI & INAN, 2011; HOFFMAN et al., 2008; ZEMBIC et al., 2009; SAILER et al.; 2009) assim como uma baixa adesão bacteriana (BREMER et al., 2011) são relatadas como fatores favoráveis para esse material. Recentes trabalhos apresentam dados com relação ao aumento da rugosidade superficial de superfícies de zircônia (ABOUSHELIB et al., 2011) para favorecer a aposição óssea na interface implante - tecido ósseo. A proliferação de osteoblastos em superfície de titânio tratado com zircônia e cálcio foi relatada com sendo superior (WANG et al., 2011).

A utilização dos lasers em implantodontia pode ser relacionada com diversas finalidades (ROMANOS et al., 2009). A superfície de titânio pode ser irradiada para favorecer a adesão de osteoblastos (ROMANOS et al., 2006). O laser de dióxido de carbono (CO_2) e o laser de érbio cromo dopado com ítrio, escândio, gálio e granada (Er,Cr:YSGG) apresentou resultados positivos para adesão de osteoblastos em superfícies de titânio tratadas (ROMANOS et al. 2006).

A peri-implantite é uma doença caracterizada pela inflamação, inchaço e sangramento de tecido mole (FROUM et al., 2011) com mudanças no nível da crista óssea em conjunto com sangramento a sondagem e/ou bolsas periimplantares (LANG et al., 2011) que podem causar a perda de implantes odontológicos particularmente devido a dificuldade em eliminar o biofilme (GONÇALVES et al., 2010). O objetivo do tratamento da peri-implantite é reduzir a adesão bacteriana enquanto deixa a superfície do implante intacta para a adsorção das células óssea regeneradoras (HAUSER-GERSPACH et al., 2011). Assim como em tratamento de periodontites, a remoção supra e sub mucosa dos depósitos bacterianos é essencial no tratamento de infecções peri-implantares (MOMBELLI et al., 1992).

A descontaminação da superfície dos implantes pode ser realizada por diversos métodos como o ultrassom, curetas manuais e o tratamento com laser (MENDONÇA et al., 2008; SCHWARZ et al. 2005; TAKASAKI et al., 2007; HAUSER-GERSPACH et al., 2010). São relatados na literatura os comprimentos de onda que proporcionam efeitos benéficos para o tratamento da peri-implantite: arsenido de gálio alumínio (laser de diodo, com comprimento de onda de 980 nm) (GEMINIANI et al., 2011b; GONÇALVES et al., 2010; KREISLER et al., 2002; HAUSER-GERSPACH et al., 2010; ROMANOS et al., 2006; STUBINGER et al., 2010) neodímio dopado com ítrio alumínio (Nd:YAG, com comprimento de onda de 1064 nm) (GONÇALVES et al., 2010; ROMANOS et al., 2000); érbio ítrio, alumínio e granada (Er:YAG) (comprimento de onda de 2940 nm) (GEMINIANI et al., 2011a, QUARANTA et al., 2009; SCHWARZ et al., 2005; STUBINGER et al., 2010; SUBASI & INAN, 2011; TAKASAKI et al., 2007); laser de dióxido de carbono (CO_2) (com comprimento de onda de 10.600 nm) (GEMINIANI et al., 2011a; HAUSER-GERSPACH et al., 2010; KREISLER et al., 2002; STUBINGER et al., 2010; STUBINGER et al. 2008); o érbio cromo dopado com ítrio, escandio, gálio e granada (Er,Cr:YSGG) com comprimento de onda de 2780 nm (AZZEH et al., 2008, MILLER, 2004; ROMANOS et al., 2006).

Danos térmicos severos são relatados em estrutura dental e tecidos circunvizinhos, como por exemplo, fendas, rachaduras e fusão que podem ser evitados com a refrigeração a água (DE MOOR & DELMÉ, 2009). Stubinger et al. (2008) relataram ausência de alteração superficial em zircônia após irradiação com o laser de Er:YAG porém, demonstraram alteração 2 mm abaixo da área irradiada. O laser de CO_2, quando usado com 2 e 4 W, afetou negativamente a superfície de implantes de zircônia com danos superficiais expressivos e alteração de composição observadas através da análise de energia dispersiva por raio-X (EDX) (STUBINGER et al., 2008). Também foi relatado áreas de “melt” ou fusão após a irradiação de Y-TZP com laser de Nd:YAG (3 e 4 W) devido a dano térmico (AKYIL et al., 2010). A irradiação com laser de Er:YAG causou alterações térmicas como “cracks” e perda de material em zircônia com 3 e 4 W porém, nenhuma alteração foi observada com parâmetros inferiores (1 e 2 W) (AKYIL et al., 2010).

As curetas periodontais utilizadas para o debridamento produzem uma superfície mais rugosa do que o tratamento com laser de Er:YAG (MENDONÇA et al., 2008). Porém, resultados histológicos favoráveis após irradiação com laser de Er:YAG promovendo uma maior re-osseointegração comparado ao grupo cureta foram relatados por Takasaki et al. (2007). A descontaminação com laser de Er:YAG é efetiva em diferentes superfícies de titânio (QUARANTA et al., 2009) sem alteração superficial observada por microscopia eletrônica de varredura. O laser de diodo é relatado como efetivo para a redução da viabilidade bacteriana (HAUSER-GERSPACH et al., 2010; GONÇALVES et al., 2010). Para o tratamento da peri-implantite a aplicação de sistemas laser não-contato é uma alternativa para evitar-se as alterações superficiais (STUBINGER et al., 2010; GONÇALVES et al., 2010).

A irradiação com laser de Er,Cr:YSGG favorece a regeneração óssea ao redor de implantes de titânio (AZZEH et al., 2008) e é altamente capaz de remover biofilme, porém ocorre uma diminuição da biocompatibilidade superficial (SCHWARZ et al. 2006). O parâmetro de 1,5 W foi descrito como suficiente para remoção de biofilme com adesão de osteoblastos (SCHWARZ et al., 2006). Sua utilização foi eficiente para remover contaminantes em superfícies rugosas de implantes de titânio sem alterações superficiais (MILLER, 2004).

O objetivo do presente estudo foi investigar os efeitos do laser Er,Cr:YSGG com parâmetro de descontaminação em diferentes condições em parâmetros de rugosidade (Sa) zircônia (Y-TZP) e titânio (TI) com jateamento de partículas grandes e ataque ácido (SLA).

2. PROPOSIÇÃO

O objetivo desse estudo foi avaliar a influência da irradiação com laser de érbio-cromo-ítrio-escândio-granada (Er,Cr:YSGG) em diferentes condições sobre a rugosidade superficial de uma zircônia odontológica e do titânio SLA por meio da análise da rugosidade (Sa, μm^2) e microscopia confocal e da caracterização da topografia de superfície.

METODOLOGIA E RESULTADOS

3. 1 Artigo

Surface alterations of a ceramic and titanium substrate after Er,Cr:YSGG irradiation

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ABSTRACT

Objectives: The study investigated the effects of erbium chromium-doped: yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser irradiation with decontamination parameter on roughness parameters (Sa, μm^2) for yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) and titanium sandblasted large grit acid-etched (SLA) titanium (TI) material. **Methods:** Fifteen disks of yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) (AS Technology Titanium FIX, São José dos Campos, Brazil) with 5 mm diameter and 3 mm high standardized with CAD-CAM were used. Ten disks of 5 mm in diameter and 4 mm in thickness of titanium (SLA) surface (AS Technology Titanium FIX, São José dos Campos, Brazil) were used. The Y-TZP zirconia disks were randomized in three groups to laser irradiation (n=5): Y-TZP_G1= control (no laser treatment); Y-TZP_G2= Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/25%); Y-TZP_G3= Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/0%). The titanium disks were randomized in two groups to laser irradiation (n=5): TI_G1= Titanium SLA control (no laser treatment); TI_G2= titanium SLA irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/25%). The material surface were analysed by confocal white light microscopy. The mean of Sa values (μm^2) and standard deviation were calculated from five profiles of each group. Values were statistically analyzed by one-way ANOVA at the 95% confidence level and compared by Tukey Hoc post-test (α=0.05) for each material. **Results:** ANOVA showed statistical significant differences for the factor “laser” (p=0.00179) for Y-TZP zirconia material. The results (μm^2) were: Y-TZP_G1: 2.589 (1.098)A; Y-TZP_G2: 0.815 (0.171)B; Y-TZP_G3: 0.746 (0.391)B. ANOVA showed statistical significant differences for the factor “laser” (p= 0.00949) for titanium SLA material. The results (μm^2) were: TI_G1: 1.990 (0.507)B; TI_G2: 3.367 (0.754)A. **Conclusions:**

Er,Cr:YSGG used on parameter of decontamination alters the roughness surface of zirconia Y-TZP and sandblasted large grit acid-etched titanium.

KEY WORDS: zirconia; titanium disks; confocal microscope; implant

INTRODUCTION

The rehabilitation of partially or totally edentulous with titanium implants reachs high values of sucess (BUSER et al., 1997; AL-NAWAS et al., 2012). It has been demonstrated the influence of implants surface characteristics as a relevent factor to obtain osseointegration (SCHWARZ et al., 2009). The machined surfaces are relatively less rough compared to the nano-micro structured that shows an increase of bone implant contact (BIC) which favours the osseointegration (D'AVILA et al., 2010; ROMANOS et al., 2006). Surface sandblasted with large grit and acid-etched (SLA) is an implant surface modification that is related to improve the osteointegration (SCHWARZ et al., 2009).

Pure zirconia can assume three crystallographic forms: monoclinic, tetragonal and cubic (DENRY & KELLY, 2008). Zirconia is the common name for zirconium dioxide (ZrO_2) (AKYIL et al., 2010) and stabilizing oxides such as Y_2O_3 allows the retention of the tetragonal structure at room temperature (DENRY & KELLY, 2008). Zirconia ceramics (yttrium-stabilized tetragonal zirconia polycrystal)(Y-TZP) has biocompatibility, and tooth-like color, inherent strength (DELGADO-RUÍZ et al., 2011; STUBINGER et al., 2008; SUBASI & INAN, 2011). Abutments of Y-TZP have become an alternative to titanium and also as dental implant material (GAHLERT et al., 2010; ZEMBIC et al., 2009; SAILER et al., 2009). It has been claimed that the zirconia surface has also decreased bacterial adhesion (BREMER et al., 2011).

Periimplantitis is a disease that causes crestal bone resorption with bleeding on probing with or without concomitant deepening of periimplant pocket (LANG et al., 2011). The decontamination of implants surface can be performed by several methods like ultrasonic system, manual curette and laser treatment (MENDONÇA et al., 2008; SCHWARZ et al., 2005; TAKASAKI et al., 2007; HAUSER-GERSPACH et al., 2010).

Several laser wavelengths have been studied to this purpose (ROMANOS et al., 2009) such as Gallium-Aluminium-Arsenide (GaAlAs, diode laser, 980 nm wavelength) (GONÇALVES et al., 2010; KREISLER et al., 2002; HAUSER-GERSPACH et al., 2010; ROMANOS et al., 2009; STUBINGER et al., 2010) Neodymium-doped Yttrium Aluminium Garnet (Nd:YAG, 1064 nm wavelength) (GONÇALVES et al., 2010; ROMANOS et al., 2000); Erbium: yttrium aluminium garnet (Er:YAG) (wavelength 2940 nm) (GEMINIANI et al., 2011, QUARANTA et al., 2009; SCHWARZ et al., 2005; STUBINGER et al., 2010; SUBASI & INAN, 2011; TAKASAKI et al., 2007); carbon dioxide laser (CO_2) (GEMINIANI et al., 2011; HAUSER-GERSPACH et al., 2010; KREISLER et al., 2002; STUBINGER et al., 2010; STUBINGER et al., 2008); erbium chromium-doped: yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) wavelength 2780 nm (AZZEH et al., 2008; MILLER, 2004; ROMANOS et al., 2006).

The Er,Cr:YSGG laser irradiation has been studied as an alternative tool to decontamination of titanium implants surface (SCHWARZ et al., 2006; ROMANOS et al., 2006; AZZEH et al., 2008) with 1.5 W and water irrigation and presented osteoblast attachment. The heat conduction is minimized with a water spray used for cooling to avoid thermal effects such as cracks and melted areas (DE MOOR & DELMÉ, 2009).

The aim of the study was to investigate the effects of erbium chromium-doped: yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser irradiation with different conditions on roughness parameters (Sa - arithmetic mean deviation of the profile and the surface) for yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) and titanium sandblasted large grit and acid etched SLA (TI) material.

2. MATERIALS AND METHODS

Experimental design

The experimental units consisted of 25 disks (n=5 per group). Fifteen disks of yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) (AS Technology Titanium FIX, São José dos Campos, Brazil) with 5 mm diameter and 3 mm high were standartized from CAD-CAM blocks. Ten disks of 5 mm in diameter and 4 mm in thickness of titanium sandblasted acid-etched surface (AS Technology Titanium FIX, São José dos Campos, Brazil) with 100 µm Al_3O_2 particles (SLA) were used. After the sandblast, the specimens were ultrasonically cleaned with an alkaline solution, washed in distilled water, pickled with HNO_3.

Specimen preparation/ Laser system

The Y-TZP zirconia disks were randomized in three groups to laser irradiation: Y-TZP_G1= control (no laser treatment); Y-TZP_G2= Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/25%); Y-TZP_G3= Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/0%) according to Table 1.

Table 1. Experimental groups of Y-TZP

Groups		Air/water (a/w) cooling settings in percentages	n= Samples
Y-TZP_G1	Y-TZP Control	-	5
Y-TZP_G2	Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W)	(a/w) 80%/25%	5
Y-TZP_G3	Y-TZP irradiated with Er,Cr:YSGG laser (1.5 W)	(a/w) 80%/0%	5

The titanium SLA disks were randomized in two groups to laser irradiation: TI_G1= Titanium SLA control (no laser treatment); TI_G2= titanium SLA irradiated with Er,Cr:YSGG laser (1.5 W; air-water cooling proportion 80%/25%) according to Table 2.

Table 2. Titanium experimental groups

Groups		Air/water (a/w) cooling settings in percentages	n= Samples
TI_G1	Titanium Control	-	5
TI_G2	Titanium irradiated with Er,Cr:YSGG laser (1.5 W)	(a/w) 80%/25%	5

Control groups did not receive any laser treatment (Y-TZP_G1 and TI_G1). Er,Cr:YSGG laser (λ=2780nm; Waterlase, Biolase Technologies Inc, Irvine, CA, USA) was used on each Y-TZP zirconia disk (Y-TZP_G2 and Y-TZP_G3) and titanium disk (TI_G2) in focused mode with a 600 µm quartz core (G4). Repetition rate was fixed on 20 Hz. The air-water cooling proportion was fixed on 80%/25% to Y-TZP_G2 and TI_G2. The air-water cooling proportion was fixed on 80%/0% to Y-TZP_G3. One single trained operator uniformily irradiated by hand in a grid pattern for 30 s the disk surface.

Confocal White Light Microscope:

The surface topography of disks was investigated using confocal microscope Leica Scan DCM 3D (Leica Microsystems Ltd, Switzerland) with objective magnification 50X, numeral aperture 0.9, optical resolution (X/Y) 0.16 µm, FOV (µm) is 254.64 X 190.90. The vertical resolution is < 3 nm and has sub-micron lateral resolution in confocal mode according to manufacturer. The control groups (TI_G1 and Y-TZP_G1) were used as references. Leica DCM 3D Dual Core profiler software (Leica Microsystems Ltd, Switzerland) calculated the surface roughness. Sa and Ra were recorded. Sa measure was performed in 254.64 X 190.90 µm^2 and Ra length of 254.64 µm (768 X 576 pixels).

Statistical analysis:

The mean of roughness values (Sa, (µm^2) and standard deviation were calculated from five profiles of each group. The factors under study for Y-TZP zirconia material were laser (at three levels): no laser treatment, laser treatment (air/water - 80%/25%) and laser treatment (air/water - 80%/0%). Values were statistically analyzed by one-way ANOVA at the 95% confidence level and compared by a Tukey Hoc post-test (α=0.05). The software employed was SANEST (EPAMIG, MG, Brazil).

The factor under study for titanium material was laser (at two levels): no laser treatment and laser treatment. Values were statistically analyzed by one-way ANOVA at the 95% confidence level and compared by a Tukey Hoc post-test (α=0.05). The software employed was SANEST (EPAMIG, MG, Brazil).

RESULTS

Table 3 shows the exploratory values for roughness parameters (Sa and Ra) for Y-TZP zirconia material.

Table 3. Effects of Er,Cr:YSGG laser irradiation for Y-TPZ material on roughness parameters (Sa and Ra).

Parameter/ treatment Y-TPZ	**n**	**Mean [standard deviation]**	**Maximum value**	**Minimum value**
Sa (μm^2)				
Control (Y-TZP_G1)	**5**	**2.589** [1.098]	**5.988**	**35.826**
1.5W/20 Hz/30s - (air/water) 80%/25% (Y-TZP_G2)	**5**	**0.815** [0.171]	**3.009**	**3.197**
1.5W/20 Hz/30s - (air/water) 80%/0% (Y-TZP_G3)	**5**	**0.746** [0.391]	**3.896**	**3.885**
Ra (μm)				
Control (Y-TZP_G1)	**5**	**2.014** [0.709]	**4.946**	**30.818**
1.5W/20 Hz/30s- (air/water) 80%/25% (Y-TZP_G2)	**5**	**0.183** [0.146]	**0.472**	**1.236**
1.5W/20 Hz/30s - (air/water) 80%/0% (Y-TZP_G3)	**5**	**0.070** [0.015]	**0.348**	**0.3238**

n=sample number

ANOVA showed statistical significant differences for the factor “laser” ($p=0.00179$) for Y-TZP zirconia material (ANEXO 1).

Table 4 shows the exploratory values for Sa roughness parameters (μm^2) and the results of Tukey test for Y-TZP zirconia material.

Table 4. Means [standard deviations] of Sa roughness parameters (μm^2) and the results of Tukey test for factor laser for Y-TZP material.

Laser Treatment	**Y-TZP_G1 (n=5)**	**Y-TZP_G2 (n=5)**	**Y-TZP_G3 (n=5)**
Y-TZP	**2.589** [1.098]**A**	**0.815** [0.171]**B**	**0.746** [0.391]**B**

Means followed by different upper case letter at row indicate statistical differences ($p< 0.05$)
n=sample number

Control group had higher Sa values than the lasers groups. The Er,Cr:YSGG laser influenced on the roughness parameter.

Table 5 shows the exploratory values for roughness parameters (Sa and Ra) for titanium SLA material.

Table 5. Effects of Er,Cr:YSGG laser irradiation for titanium SLA material on roughness parameters (Sa and Ra).

Parameter/ treatment Titanium SLA	**n**	**Mean [standard deviation]**	**Maximum value**	**Minimum value**
Sa (μm^2)				
Control (TI_G1)	**5**	**1.990** [0.507]	**4.148**	**32.047**
1.5W/20 Hz/30s - (air/water) 80%/25% (TI_G2)	**5**	**3.367** [0.754]	**11.468**	**12.584**
Ra (μm)				
Control (TI_G1)	**5**	**1.777** [0.530]	**3.379**	**26.911**
1.5W/20 Hz/30s- (air/water) 80%/25% (TI_G2)	**5**	**3.840** [0.623]	**12.505**	**7.308**

ANOVA showed statistical significant differences for the factor “laser” (p= 0.00949) for titanium material (ANEXO 2).

Table 6 shows the exploratory values for Sa roughness parameters (μm^2) and the results of Tukey test for factor laser for titanium material.

Table 6. Means [standard deviations] of Sa roughness parameters (μm^2) and the results of Tukey test for factor laser for titanium material.

Laser Treatment	**TI_G1 (n=5)**	**TI_G2 (n=5)**
Titanium	**1.990** [0.507]**B**	**3.367** [0.754]**A**

Means followed by different upper case letter at row indicate statistical differences ($p < 0.05$)
n=sample number

Titanium SLA material had higher Sa values after laser treatment. The Er,Cr:YSGG laser influenced on the roughness parameter.

Figure 1 shows one representative 2D and 3D image obtained for Y-TZP zirconia material (Y-TZP_G1: control group).

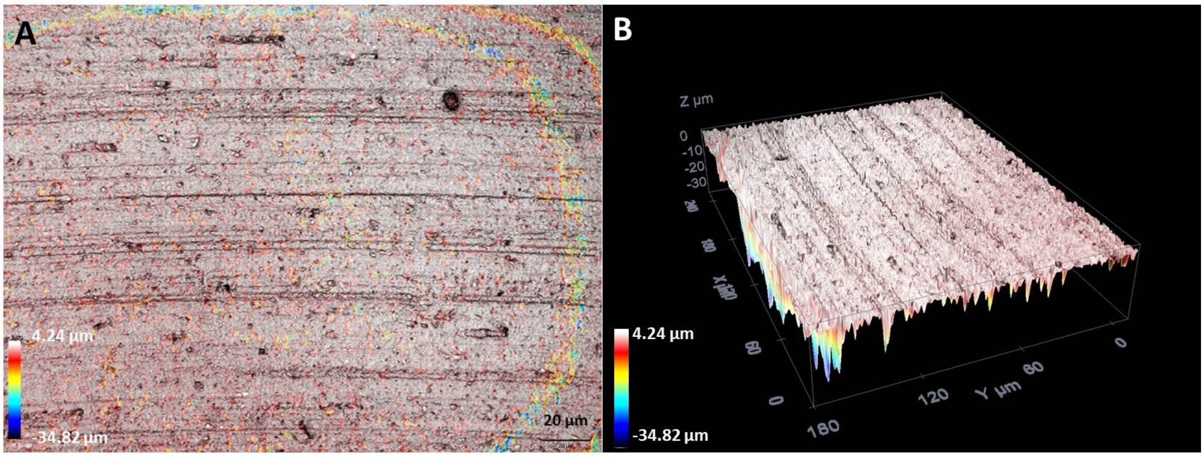

Figure 1. Representative 2D image obtained for Y-TZP zirconia material (Y-TZP_G1: control group) (A). Representative 3D image obtained for Y-TZP zirconia material (Y-TZP_G1: control group) (B) (Bar: 20 µm) (50X)

It was observed that the mean Sa roughness value for Y-TZP_G1 group was 2.589 µm^2 (Table 3 and 4).

Figure 2 shows one representative 2D and 3D image obtained for titanium SLA material (TI_G1: control group).

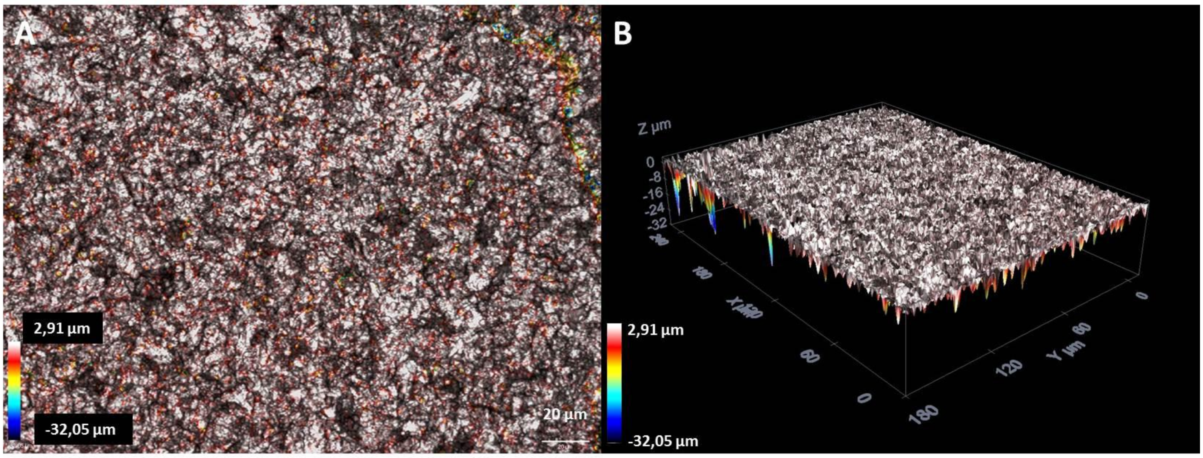

Figure 2. Representative 2D image obtained for titanium SLA material (TI_G1: control group) (A). Representative 3D image obtained for titanium SLA material (TI_G1: control group) (B) (Bar: 20 µm) (50X)

It was observed that the mean Sa roughness value for TI_G1 group was 1.990 µm^2 (Table 5 and 6).

Figure 3 shows one representative 2D and 3D image obtained for irradiated Y-TZP material (Y-TZP_G2: Er,Cr:YSGG, air/water 80%/25%).

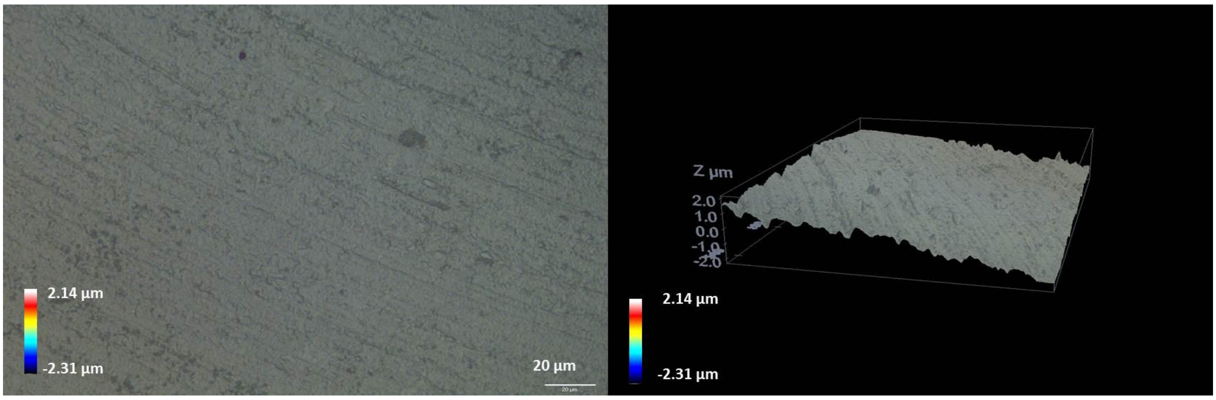

Figure 3. Representative 2D image obtained for irradiated Y-TZP zirconia material (Y-TZP_G2: Er,Cr:YSGG, air/water 80%/25%)(A). Representative 3D image obtained for irradiated Y-TZP zirconia material (Y-TZP_G2: Er,Cr:YSGG, air/water 80%/25%)(B). (Bar: 20 µm) (50X)

It was observed that the mean Sa roughness value for Y-TZP_G2 group was 0.815 μm^2 (Table 3 and 4).

Figure 4 shows one representative 2D and 3D image obtained for irradiated Y-TZP material (Y-TZP_G3: Er,Cr:YSGG, air/water 80%/0%).

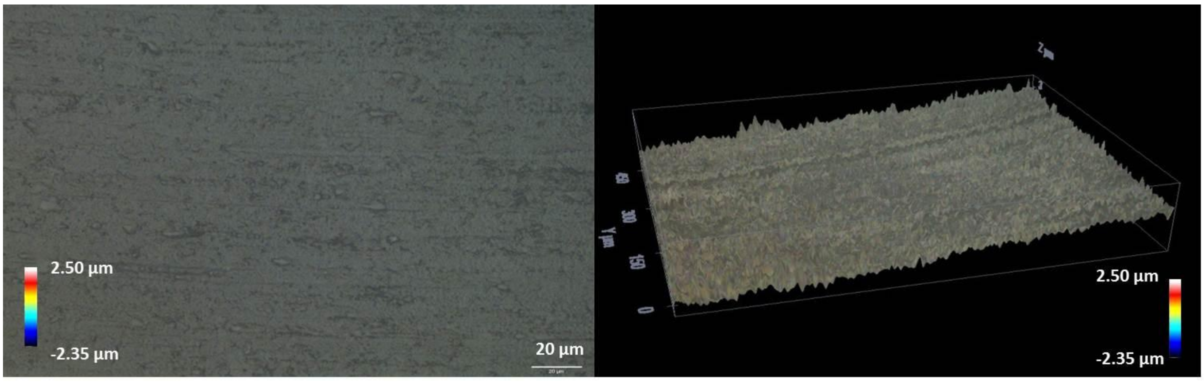

Figure 4. Representative 2D image obtained for irradiated Y-TZP zirconia material (Y-TZP_G3: Er,Cr:YSGG, air/water 80%/0%)(A). Representative 3D image obtained for irradiated Y-TZP zirconia material (Y-TZP_G3: Er,Cr:YSGG, air/water 80%/0%)(B). (Bar: 20 µm) (50X)

It was observed that the mean Sa roughness value for Y-TZP_G3 group was 0.746 μm^2 (Table 3 and 4).

Figure 5 shows one representative 2D and 3D image obtained for irradiated titanium material (TI_G2: Er,Cr:YSGG).

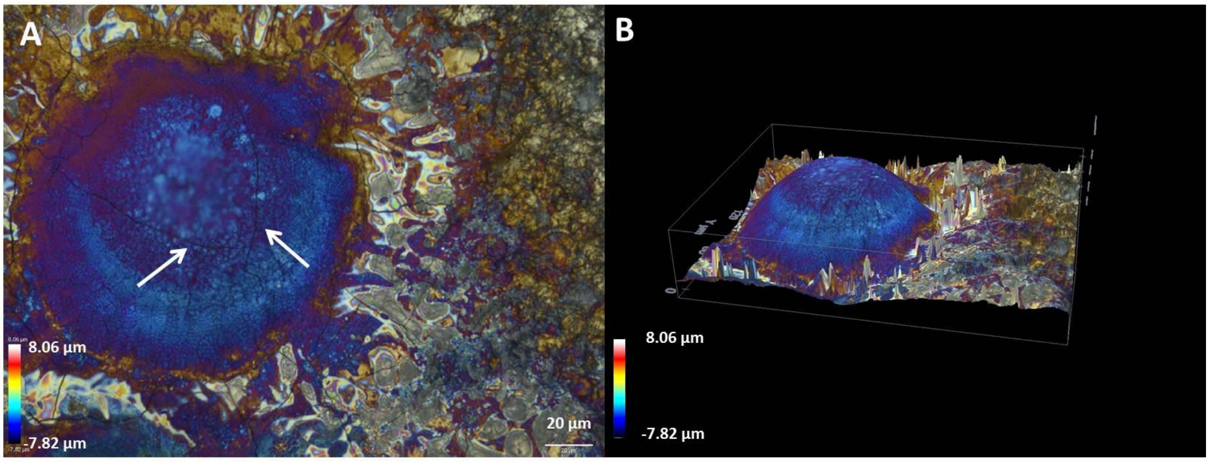

Figure 5. Representative 2D image obtained for irradiated titanium material (TI_G2: Er,Cr:YSGG)(A).There is evidence of thermal damage with a projection and cracks on surface (arrows). Representative 3D image obtained for irradiated titanium material (TI_G2: Er,Cr:YSGG)(B). (Bar: 20 µm) (50X)

It was observed that the mean Sa roughness value for TI_G2 group was 3.367 μm^2 (Table 5 and 6). There were cracks on surface and projections with 8 µm high on titanium surface were evident.

DISCUSSION

Peri-implant infection is a concern because its progression can cause implant lost (LANG et al., 2011). The plaque biofilm may alter the titanium implant surface characteristics. It has been suggested that the bacterial contamination of the surface may affects the dioxide layer (SCHWARZ et al., 2006). Biofilms increase the amount of carbon at the dioxide layer (SCHWARZ et al., 2009).

It has been described thermal alterations on titanium SLA such as melting after Nd:YAG (ROMANOS et al., 2000) laser irradiation. STUBINGER et al. (2010) analyzed at scanning electron microscopy the Er: YAG irradiated with titanium SLA surface and reported that the surface presented melted areas.

AKYIL et al. (2010) on a study aiming the increase Y-TZP roughness to improve resin cement adhesion reported surface thermal alterations. They described melted areas on Y-TZP surface after Nd:YAG (3 and 4 W) due to thermal damage.

Er:YAG laser irradiation also caused thermal alterations such as craks and loss of zirconia despite no surface alterations was observed in lower parameters of the same wavelength.

The association of microorganisms know from chronic periodontitis (SCHWARZ et al., 2006) are present at peri-implantitis sites and must be removed. At this point, the Er,Cr:YSGG laser irradiation can be used to decontaminate the titanium implants surface (SCHWARZ et al., 2006; ROMANOS et al., 2006) and the 1.5 W parameter was selected in the present study based upon the osteoblast attachment like described by ROMANOS et al. (2006) and SCHWARZ et al. (2006). On the other hand, a recent study indicated that Er:YAG laser can alter the titanium implants surface with a negative effect on the viability and activity of osteoblastic cells (GALLI et al., 2011) at 200 mJ/10 Hz.

The titanium material has been demonstrated to interact with laser treatment in different ways depending of the surface. The interaction of Er,Cr:YSGG to titanium plasma-sprayed showed no superficial alteration (MILLER, 2004) at the highest power setting (6 W), however the present study showed superficial alterations with lower power setting (1.5 W) and statistical differences compared to the control group showing an increase in roughness and topographic changes such as cracks. The titanium surface presented visual alteration and the confocal microscopy images confirmed these changes. The irradiated area presented projections with aproximately 8 μm high with concomitant cracks at material surface besides titanium surface received water cooling.

A study from SCHWARZ et al. (2006) evaluated the decontamination of titanium SLA surface with Er,Cr:YSGG at 1.5 W/20 Hz with air/water proportion of 50%/50%. Besides the very close methodology with the present study, the authors related no thermal effect such as melting or lost of porosity. The laser was applied contact-mode and the present study used focused mode. This fact can explain the morphological alterations and increased roughness. ROMANOS et al. (2006) also related no changes on titanium surface with1.25 W/ 20Hz with air/water proportion of 42%/41% but they did not describe the aplication mode. The factors related to the operative procedure are important: irradiation time, water cooling and irradiation distance (DE MOOR & DELMÉ, 2009).

The clinical application of the Er,Cr:YSGG laser to decontaminate implant surface is different of the *in vitro* situation because in oral cavity there is the presence of water of the gingival fluid, saliva and blood. The wavelength of Er,Cr: YSGG laser is highly specific to water and the behavior of the laser treatment to decontaminate superficial implants can be different on clinical situation. The good clinical results presented by AZZEH et al. (2008) corroborate with these but the authors did not specify the titanium surface irradiated.

The zirconia material is widely used in biomedical area (HISBERGUES et al., 2009). It presents lower bacterial adhesion and bacterial biofilm formation compared to other current dental material (BREMER et al., 2011). STUBINGER et al. (2008) analyzed at scanning electron microscopy the Er: YAG irradiated zirconia surface with several settings parameters and found no superficial alteration including the energy-dispersive X-ray (EDX). They found no differences after confocal 3D white light microscopy on roughness parameters. The present study did not agree with those findings because the Y-TZP showed decreased on roughness and concomitant 3D alteration that can be visualized at Figure 3B and 4B (with or without cooling). The thermal effect probably affected the zirconia and induced to reverse fase transformation monoclinic to tetragonal (DENRY & KELLY, 2008) but this assumption could be only validated after X-ray diffraction (XRD) analysis.

The cooling is crucial to the laser safety (DE MOOR & DELMÉ, 2009) but the present study selected a critical clinical situation without cooling to compare the superficial changes to Y-TZP surface. The cooling was not the effect that changed the superficial because there were not significant differences on roughness parameters or on 3D image.

To the best of our knowledge there are no comparable studies and it is necessary further analysis especially of the osteoblastic behavior at laser modified surfaces and its clinical use.

CONCLUSION

- The Y-TZP surface were altered by the Er,Cr:YSGG laser with decontamination parameter.
- After Er,Cr:YSGG laser irradiation the zirconia showed lower surface roughness.

- Compared to the refrigerated group no difference was observed on laser Er,Cr:YSGG non-refrigerated Y-TZP group.
- Titanium SLA irradiated with Er,Cr:YSGG laser showed an increase on surface roughness compared to the non-irradiated group and with presence of superficial cracks.

DISCLOSURE

The authors have no interest in any of the companies or products mentioned in this article.

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4. CONCLUSÕES

Através dos resultados obtidos e, considerando-se as condições experimentais deste estudo, pode-se concluir que:

- A rugosidade superficial das amostras de Y-TZP foi alterada após o tratamento com laser de Er,Cr:YSGG.

- Após irradiação com laser de Er,Cr:YSGG a superfície da zircônia apresentou menor rugosidade superficial.

- A remoção da irrigação do laser de Er,Cr:YSGG não causou diferenças quando comparadas ao grupo com refrigeração no material Y-TZP.

- Após irradiação com laser de Er,Cr:YSGG a superfície de titânio jateado e condicionado com ácido apresentou rugosidade superficial maior do que a rugosidade do grupo não irradiado com a presença de fraturas superficiais.

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ANEXO 1- Análise estatística para os resultados rugosidade para Y-TZP

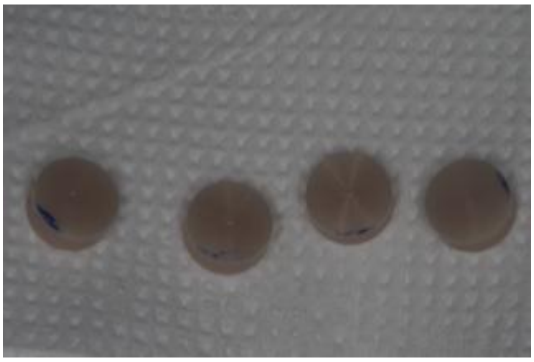
```
           ***********************************************************
        *          SANEST - SISTEMA DE ANALISE ESTATISTICA         *
        *   Autores: Elio Paulo Zonta  -  Amauri Almeida Machado   *
        *         Instituto Agronomico de Campinas - I A C         *
        *      ANALISE DA VARIAVEL RUGOSIDA - ARQUIVO: ZRERCR      *
        ************************************************************

                          CODIGO DO PROJETO:

                             RESPONSAVEL: ALE

                         DELINEAMENTO EXPERIMENTAL:

                        OBSERVACOES NAO TRANSFORMADAS

                             NOME DOS FATORES
                            ------------------
                             FATOR     NOME
                            ------------------
                               A     LASER
                            ------------------


                        QUADRO DA ANALISE DE VARIANCIA
---------------------------------------------------------------------------------------------
 CAUSAS DA VARIACAO     G.L.           S.Q.                Q.M.         VALOR F   PROB.>F
---------------------------------------------------------------------------------------------
  LASER                   2         10.9090573          5.4545287      11.7875   0.00179
  RESIDUO                12          5.5528395          0.4627366
---------------------------------------------------------------------------------------------
  TOTAL                  14         16.4618969
---------------------------------------------------------------------------------------------

 MEDIA GERAL =       1.383407

 COEFICIENTE DE VARIACAO  =   49.172 %


                    TESTE DE TUKEY  PARA MEDIAS DE LASER
   ---------------------------------------------------------------------------------
    NUM.ORDEM  NUM.TRAT.   NOME    NUM.REPET.     MEDIAS     MEDIAS ORIGINAIS  5%  1%
   ---------------------------------------------------------------------------------
        1         1                   5        2.588800        2.588800      a   A
        2         2     LASER         5        0.815000        0.815000       b   B
        3         3     LASEZERO      5        0.746420        0.746420       b   B
   ---------------------------------------------------------------------------------
 MEDIAS SEGUIDAS POR LETRAS DISTINTAS DIFEREM ENTRE SI AO NIVEL DE SIGNIFICANCIA INDICADO
```

ANEXO 2- Análise estatística para os resultados de rugosidade para titânio SLA

```
                      *************************************************************
                      *          SANEST - SISTEMA DE ANALISE ESTATISTICA          *
                      *   Autores: Elio Paulo Zonta  -  Amauri Almeida Machado    *
                      *         Instituto Agronomico de Campinas - I A C          *
                      *      ANALISE DA VARIAVEL RUGOSIDA - ARQUIVO: TITANI       *
                      *************************************************************

                                        CODIGO DO PROJETO:

                                            RESPONSAVEL:

                                      DELINEAMENTO EXPERIMENTAL:

                                     OBSERVACOES NAO TRANSFORMADAS

                                           NOME DOS FATORES
                                          ------------------
                                           FATOR     NOME
                                          ------------------
                                             A     LASER
                                          ------------------


                               QUADRO DA ANALISE DE VARIANCIA
---------------------------------------------------------------------------------------------
 CAUSAS DA VARIACAO     G.L.           S.Q.                Q.M.         VALOR F   PROB.>F
---------------------------------------------------------------------------------------------
  LASER                   1           4.7423919           4.7423919     11.4810   0.00949
  RESIDUO                 8           3.3045063           0.4130633
---------------------------------------------------------------------------------------------
  TOTAL                   9           8.0468982
---------------------------------------------------------------------------------------------

 MEDIA GERAL =       2.678550

 COEFICIENTE DE VARIACAO  =   23.994 %


                           TESTE DE TUKEY  PARA MEDIAS DE LASER
   ------------------------------------------------------------------------------------
    NUM.ORDEM  NUM.TRAT.   NOME    NUM.REPET.     MEDIAS     MEDIAS ORIGINAIS  5%  1%
   ------------------------------------------------------------------------------------
        1          2     LASER         5         3.367200        3.367200      a   A
        2          1     CONTROL       5         1.989900        1.989900       b   B
   ------------------------------------------------------------------------------------
 MEDIAS SEGUIDAS POR LETRAS DISTINTAS DIFEREM ENTRE SI AO NIVEL DE SIGNIFICANCIA INDICADO
                             ---------------------
```

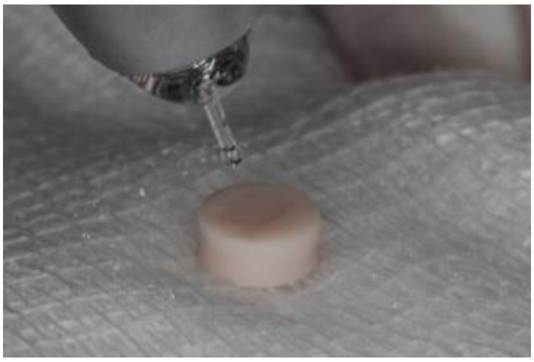
ANEXOS

Anexo 3: Discos de zircônia (Y-TZP) antes da irradiação com Er,Cr:YSGG.



Anexo 4: Disco de titânio (SLA) antes da irradiação Er,Cr:YSGG

Anexo 5: Irradiação da zircônia (Y-TZP) com laser Er,Cr:YSGG com refrigeração ar/água 80%/20%.



Anexo 6: Irradiação do titânio (SLA) com laser Er,Cr:YSGG com refrigeração ar/água 80%/20%.

Anexo 7: Disco de titânio (SLA) após irradiação com laser Er,Cr:YSGG .

Anexo 8: Disco de zircônia (Y-TZP) após irradiação com laser Er,Cr:YSGG.