UNIVERSIDADE DE UBERABA
MESTRADO ACADÊMICO EM ODONTOLOGIA

GABRIELLA RODOVALHO PAIVA

INFLUÊNCIA DO *LASER* Er,Cr:YSGG, ASSOCIADO OU NÃO AO VERNIZ FLUORETADO 5%, NA RUGOSIDADE SUPERFICIAL E NA PERDA DE VOLUME DA DENTINA RADICULAR BOVINA SUBMETIDA A DESAFIOS EROSIVOS E/OU ABRASIVOS

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia - Mestrado Acadêmico da Universidade de Uberaba, como requisito para obtenção do título de Mestre em Odontologia, na Área de Concentração em Clínica Odontológica Integrada.

Orientador: Prof. Dr. Cesar Penazzo Lepri.

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DEDICATÓRIA

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RESUMO

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O objetivo desse estudo *in vitro* foi avaliar a influência do laser Er,Cr:YSGG, associado ou não ao verniz fluoretado 5%, na rugosidade superficial e na perda de volume da dentina radicular bovina submetida a desgastes erosivos e/ou abrasivos por meio da microscopia confocal de varredura a laser. Foram confeccionados 120 espécimes e divididos de acordo com os tratamentos preventivos, sendo: G1= sem tratamento preventivo (STP), G2= verniz fluoretado 5% (VF); G3=irradiação com laser Er,Cr:YSGG (L) - 0,5W, 5,0Hz, 6,25J/cm^2, 55% de ar, sem refrigeração, a uma distância de 1,0mm, durante 10 segundos de irradiação no modo varredura e G4= verniz fluoretado 5% associado ao laser Er,Cr:YSGG (VF+L). Os espécimes foram subdivididos em 03 subgrupos (n=10), de acordo com o tipo de desgaste, sendo: 1- erosão (E); 2- abrasão (A); 3- erosão seguida de abrasão (E+A). A solução erosiva utilizada foi um refrigerante à base de cola (pH=2,42 à 4ºC), com duração de 5 minutos cada ciclo erosivo. Estes desafios foram realizados duas vezes ao dia, com intervalos de 2 horas entre eles, durante 10 dias. O desgaste abrasivo, quando realizado, foi através de ensaios de escovação, 1 hora após a realização do segundo desafio erosivo diário. Os espécimes foram escovados com escova elétrica (1600 oscilações/s), carga de 2,0N, juntamente com solução *slurry*, preparada com dentífricio e água deionizada na proporção de 1:2 em peso, durante 60 segundos. Entre os ciclos, os espécimes foram mantidos em saliva artificial a 37ºC. Os dados obtidos foram analisados quanto à distribuição (Kolmogorov-Smirnov) e homogeneidade (Levene) e posteriormente submetidos à Análise de Variância (ANOVA) a um critério e pós-teste de Tukey para as comparações múltiplas de rugosidade superficial. Para a perda de volume, os dados foram submetidos ao teste de Kruskal-Wallis seguido do pós-teste de Dunn. Todos os testes estatísticos adotaram nível de significância de 5% (α=0,05). Para a área controle, todos os subgrupos apresentaram menores valores de rugosidade superficial, sem diferenças significantes entre eles *(p>0,05)*. Na área experimental, o valor de rugosidade superficial do subgrupo controle negativo [(STP) + (E+A)] foi o mais elevado (5,712µm^2 ±0,163µm^2) com diferença significante quando comparado aos demais subgrupos *(p<0,05)*. L e (VF + L) apresentaram semelhança estatística para a rugosidade superficial independentemente do tipo de desgaste (*p>0,05)*. Quanto a perda de volume, (VF+L) apresentou os menores valores, independentemente dos desgastes realizados, sendo: [(VF + L) + (E)] = 7,5%, [(VF+L) + (A)] = 7,3% e [(VF+L) + (E + A)] = 8,1% *(p>0,05)*. O subgrupo [(STP) + (E+A)] apresentou o maior valor de perda de volume (52,3%), com diferença dos demais subgrupos (p<0,05). Conclui-se que os tratamentos propostos foram eficazes na prevenção e no controle do aumento da rugosidade superficial. Para a perda de volume, a irradiação laser apresentou resultados satisfatórios, notadamente quando associada à aplicação prévia de verniz fluoretado.

PALAVRAS - CHAVES: Erosão dentária. Verniz fluoretado. Abrasão dentária. Prevenção. Laser YSGG.

ABSTRACT

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The objective of this *in vitro* study was to evaluate how the Er,Cr:YSGG laser, associated or not with 5% fluoride varnish, influence the surface roughness and volume loss of bovine root dentin submitted to erosive and/or abrasive wear by means of confocal laser scanning microscopy. There were 120 specimens made and divided according to preventive treatments: G1 = without preventive treatment (WPT); G2 = 5% fluoride varnish (FV); G3 = Er,Cr:YSGG (L) laser irradiation - 0.5 W, 5.0 Hz, 6.25 J/cm^2, 55% air, without refrigeration, at a distance of 1.0 mm, with irradiation in the scanning mode for 10 seconds; and G4 = 5% fluoride varnish combined with the Er,Cr:YSGG laser (FV + L). The specimens were subdivided into 3 subgroups (n = 10), according to the type of wear: 1 = erosion (E); 2 = abrasion (A); and 3 = erosion followed by abrasion (E + A). The erosive solution used was a cola-based soft drink (pH = 2.42 at 4º C) and each erosive cycle lasted 5 minutes. These challenges were performed twice a day, with a 2-hour interval between them, for 10 days. When performed, abrasive wear involved a 60-second brushing test performed 1 hour after the second daily erosive challenge. The specimens were brushed with an electric brush (1600 oscillations/s) at a load of 2.0 N with a slurry solution prepared at a 1:2 proportion of toothpaste and deionized water by weight. Between cycles, specimens were kept in artificial saliva at 37º C. The data obtained were analyzed for distribution (Kolmogorov-Smirnov) and homogeneity (Levene) and subsequently submitted to an analysis of variance (one-way ANOVA) and Tukey's post-hoc for multiple comparisons as regards the surface roughness. To assess volume loss, the data were submitted to the Kruskal-Wallis test followed by the Dunn post-hoc. All statistical analyzes adopted a 5% significance level ($\alpha = 0.05$). For the control area, all subgroups showed lower values of surface roughness, with no significant differences between them ($p>0.05$). On the experimental surface, the roughness value of the negative control subgroup [(WPT) + (E + A)] was significantly higher (5.712 µm^2 ± 0.163 µm^2) than the other subgroups ($p < 0.05$). The L and (FV + L) groups had statistically similar surface roughness, regardless of the type of wear ($p>0.05$). The (FV + L) group had the lowest volume loss values, regardless of the type of wear performed: [(FV + L) + (E)] = 7.5%, [(FV + L) + (A)] = 7.3%, and [(FV + L) + (E + A)] = 8.1% ($p>0.05$). The [(WPT) + (E + A)] subgroup had the highest volume loss (52.3%), which was significantly different from the other subgroups ($p<0.05$). The proposed treatments, therefore, were effective in preventing and controlling increased surface roughness. Laser irradiation showed satisfactory results for volume loss, notably when associated with the application of fluoride varnish beforehand.

KEYWORDS: Dental erosion. Fluoride varnish. Dental abrasion. Prevention. YSGG laser.

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1. INTRODUÇÃO

1INTRODUÇÃO

O aumento da expectativa de vida e o aprimoramento de técnicas preventivas oferecidas pela odontologia tem contribuído para uma maior longevidade e manutenção dos dentes naturais na cavidade oral (HELLYER, 2011). Com isso, o número de lesões cervicais não cariosas (LCNC's) aumentou gradativamente, devido às mudanças comportamentais e aos hábitos da população (YANG *et al.*, 2016; CARVALHO *et al.*, 2016).

As LCNC's surgem devido à perda de estrutura na junção cemento-esmalte sem envolvimento bacteriano, sendo caracterizadas pela perda irreversível de esmalte e dentina (AW et al., 2002; BRANDINI *et al.*, 2011) resultando na exposição da superfície radicular podendo estar associada a erosão dentária, a abrasão, a abfração e atrição, sendo, portanto, sua etiologia de origem multifatorial (GRIPPO *et al.*, 2012; LOPES e ARANHA, 2013; HASHIM *et al.*, 2014). Os tratamentos periodontais, as injúrias mecânicas, como a escovação, tratamentos cirúrgicos, aumento do consumo de alimentos ácidos e recessão gengival estão entre os fatores determinantes para o desenvolvimento de LCNC's (GRIPPO *et al.*, 2012; GERALDO- MARTINS *et al.*, 2014; BIAGI *et al.*, 2015). Além disso, os fatores etiológicos potenciais combinados, como a erosão e abrasão dentária para o desenvolvimento das LCNC's podem levar a hipersensibilidade dentinária cervical (HDC) e maior acúmulo de biofilme na região (TEIXEIRA *et al.*, 2018).

A erosão dentária pode ser de origem endógena ou exógena (MAGALHÃES *et al.*, 2014; BAUMANN *et al.*, 2017). O elevado consumo de frutas e sucos cítricos, refrigerantes, álcool, comprimidos de vitamina C e bebidas isotônicas, devido ao baixo pH e alto potencial erosivo podem acometer a estrutura dentária e provocar a erosão extrínseca, cujo tratamento é complexo devido às constantes exposições a desafios ácidos (LUSSI *et al.*, 2012; CARVALHO *et al.*, 2016; OSTROWSKA et al., 2016; ALEXANDRIA *et al.*, 2017). A erosão intrínseca é caracterizada pela mudança de pH estimulada pelo ácido clorídrico presente no estômago, o qual atinge a cavidade bucal por meio de vômitos, regurgitação ou refluxo gastroesofágico, seja em casos de bulimia, anorexia ou hérnia de hiato (WEST *et al.*, 2012; HERMONT *et al.*, 2014; CARVALHO *et al.*, 2016).

Quando há exposição de dentina na cavidade oral, há relatos de sintomatologia dolorosa, caracterizada como uma dor aguda, de início rápido e de curta duração, caracterizando a HDC, sendo a queixa mais comum entre os indivíduos com dentes acometidos por LCNC's (LOPES e ARANHA, 2013).

Neste contexto, a escovação dentária é considerada um evento de abrasão, mas sua influência na progressão das LCNC's ainda é controversa. Segundo Shellis e Addy (2014), sob condições normais e adequadas, escovar os dentes utilizando dentifrício causaria desgaste mínimo da dentina ao longo da vida. A força aplicada durante a escovação também pode agravar a progressão do desgaste das estruturas (BRANDINI *et al.*,2011; GRIPPO *et al*., 2012).

Os tratamentos preventivos para erosão dentária são direcionados contra os fatores causais e podem incluir intervenção na dieta e redução do consumo de bebidas ácidas. Pode-se também realizar alterações na superfície do dente para aumentar sua resistência a desafios ácidos; nesse sentido, o uso de fluoretos também se mostrou eficaz (MAGALHÃES *et al*., 2014; SAKAE *et al*., 2018).

As soluções contendo flúor promovem um efeito protetor, já demonstrado em estudos *in vitro e in situ* (MAGALHÃES *et al*., 2011; SCHLUETER *et al*., 2012). A aplicação tópica desses agentes fluoretados (tais como o fluoreto de sódio - NaF) podem proteger a estrutura dental contra a erosão dentária através da formação de uma camada de fluoreto de cálcio (CaF_2) na superfície (MAGALHÃES *et al*., 2011).

Gaffar (1999) utilizou o verniz fluoretado Duraphat® para tratar a HD (hipersensibilidade dentinária) e observou a formação de cristais de fluoreto de cálcio, os quais promoveram a obliteração dos túbulos dentinários, promovendo efeito terapêutico mais duradouro.

Outra alternativa no tratamento da HD é a irradiação laser, que além de promover alívio imediato da dor e a longo prazo quando comparado aos agentes tópicos dessensibilizantes convencionais, embora mais caro, oferece resultados mais rápidos, devido ao menor tempo de tratamento (BIAGI *et al.,* 2015).

Com a introdução dos lasers na Odontologia, diferentes procedimentos podem ser realizados como para preparos cavitários, tratamentos cirúrgicos, remoção de restaurações, remoção de cárie, tratamentos de superfície, terapias de clareamento e para tratamentos de HD (HASHIM *et al*., 2014). Sabe-se que os lasers de alta potência são geralmente utilizados em tratamentos cirúrgicos e periodontais. O Er,Cr:YSGG (érbio, cromo: dopado com ítrio, escândio, gálio e granada) com comprimento de onda de 2,78µm tem afinidade pela água e pelos íons hidroxila, em que a energia do laser é absorvida pelos componentes do tecido dentinário – colágeno, hidroxiapatita e água, devido a seu espectro de absorção (FRIED, 2005).

O laser Er,Cr:YSGG foi investigado para fins preventivos, embora geralmente sejam aplicados à preparação de cavidades devido ao mecanismo de ablação. No entanto, como opção preventiva, é importante que o laser não elimine a superfície tratada, mas altere o tecido morfologicamente ou quimicamente. Portanto, para alcançar o efeito preventivo, estudos foram realizados com baixas densidades de energia (parâmetros sub-ablativos) a fim de promover aumento da resistência ácida do tecido dental (HOSSAIN *et al*.,2001; FREITAS *et al*.,2010, GERALDO- MARTINS *et al.*,2014)

Stern *et al.* (1972) demonstraram pela primeira vez a possibilidade de aumentar a resistência ácida dos tecidos duros dentais após a irradiação com laser de CO_2, pois a irradiação promove aumento do conteúdo mineral e alteração da temperatura da superfície para que haja alterações morfológicas (GERALDO-MARTINS *et al*., 2013; MOURA *et al*., 2018).

A aplicação do laser Er,Cr:YSGG na superfície dental provoca aumento da temperatura superficial e altera sua estrutura química, deixando a superfície menos solúvel (FREITAS *et al*., 2010). Desta forma, estudos demonstraram que a terapia utilizando o laser promove redução efetiva da HD em comparação com sistemas dessensibilizantes convencionais (SCHWARZ *et al*., 2002; EHLERS *et al.*, 2012).

Em relação à temperatura, há preocupação quanto aos possíveis danos pulpares. Nesse sentido, Zach e Cohen (1965) observaram que se o aumento da temperatura na câmara pulpar fosse inferior a 5,5ºC não promoveria danos ao tecido pulpar saudável, pois, o esmalte e dentina não são bons condutores elétricos, o que torna a irradiação laser uma opção segura de tratamento preventivo (HE *et al*., 2011).

Raucci-Neto *et al.* (2015*)* verificaram que a irradiação com laser não causou aumento de temperatura acima de 5ºC mesmo considerando sua utilização para remoção de dentina sadia ou desmineralizada, independentemente da densidade de energia utilizada. O mesmo já havia sido observado por Geraldo- Martins *et al*. (2005), em que o laser Er:YAG promoveu aumento de temperatura aceitável no interior da câmara pulpar, sem promover danos ao tecido.

Arantes *et al.* (2018) mostraram que a associação da irradiação laser Er,Cr:YSGG com verniz fluoretado Duraphat® foi mais eficaz no tratamento preventivo da HD em comparação a utilização apenas do verniz fluoretado, pois a incorporação de íons na superfície da dentina radicular bovina foi potencializada com associação dos tratamentos, tornando a superfície mais resistente aos ácidos.

A irradiação com laser Er:YAG (érbio: dopado ítrio, alumínio e granada) associado ou não agente fluoretado foi utilizada por Scatolin *et al*. (2018) como tratamento preventivo contra desgaste abrasivo no esmalte dentário. Os resultados mostraram que o tratamento proposto, somente quando associado ao agente fluoretado, promoveu alterações morfológicas na estrutura do esmalte, proporcionando maior retenção de CaF_2, o que auxiliou no controle dos desgastes abrasivos e espera-se que haja esse mesmo comportamento para a dentina.

Desse modo, as análises de rugosidade superficial e perfilometria por meio de microscopia confocal de varredura a laser são os parâmetros mais utilizados para quantificar e descrever as alterações superficiais dos substratos dentais (ARANTES *et al.*, 2018), pois a presença de irregularidades acarreta maior acúmulo de biofilme aumentando as chances de desenvolver lesões cariosas e problemas periodontais (LEPRI e PALMA-DIBB, 2012).

Observou-se que os trabalhos científicos relacionados ao emprego do laser na Odontologia Restauradora (GERALDO-MARTINS *et al*., 2014; SCATOLIN *et al.*, 2018), especificamente no tratamento preventivo da HDC, estudaram as seguintes variáveis: erosão e abrasão. Porém, baseado na complexidade de fenômenos que se inter-relacionam e estão envolvidos no surgimento das LCNC’s nota-se a ausência de estudos que verificaram o potencial erosivo associado ao abrasivo na dentina.

Para isso, estudos laboratoriais são importantes para a definição dos protocolos de tratamento para posterior aplicação clínica. Embora muitos pacientes apresentem alto nível de erosão dentária, faltam conhecimentos sobre os tratamentos preventivos para erosão, abrasão e erosão/abrasão sendo necessário a realização de estudos laboratoriais. Diante disso, o presente estudo analisou a irradiação do laser Er,Cr:YSGG, juntamente com a aplicação do verniz fluoretado 5%, na prevenção de desgaste das estruturas dentais. Avaliou-se a rugosidade superficial e a perda de volume da dentina radicular bovina, submetida a diferentes tipos de desgaste, simulados com o uso de solução à base de cola e/ou por meio da escovação dentária.

2. PROPOSIÇÃO

2 PROPOSIÇÃO

O objetivo deste estudo *in vitro* foi avaliar a influência do laser Er,Cr:YSGG, associado ou não ao verniz fluoretado 5%, na rugosidade superficial e na perda de volume da dentina radicular bovina submetida a desgastes erosivos e/ou abrasivos.

3. CAPÍTULO 1

3 CAPÍTULO 1

Influence of the Er,Cr:YSGG laser, with or without a 5% fluoride varnish, as preventive treatment of bovine root dentin submitted to erosive and/or abrasive challenges

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Concise Title: Influence of the Er,Cr:YSGG laser on root dentin submitted to erosive and/or abrasive challenges

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4 ABSTRACT

This study evaluated how the Er,Cr:YSGG laser, associated or not with 5% fluoride varnish, influence the surface roughness and volume loss of bovine root dentin submitted to erosive and/or abrasive wear. Altogether, 120-dentin specimens were divided into groups: G1=without preventive treatment (WPT), G2=5% fluoride varnish (FV); G3=Er,Cr:YSGG (L) laser irradiation and G4=varnish combined with the laser (FV+L). The specimens (n=10) were subdivided into: 1=erosion (E); 2=abrasion (A); and 3=erosion followed by abrasion (E+A). The erosive solution used was a soft-drink (pH=2.42 at 4ºC) applied in 5-minute cycle twice a day for 10 days. Abrasive wear involved brushing for 60 seconds with an electric brush (1600-oscillations/s), at a load of 2.0N. Roughness data were submitted to a one-way ANOVA and the Tukey post-hoc ($\alpha=5\%$). For volume loss, the Kruskal-Wallis and Dunn's post-hoc ($\alpha=5\%$) were used. The lowest values of surface roughness were found in the control areas of all subgroups ($p>0.05$). In the experimental area, the [(WPT)+(E+A)] subgroup had a significantly higher roughness ($5.712\mu m^2 \pm 0.163\mu m^2$) than the other subgroups ($p<0.05$). The L and (FV+L) groups had statistically similar surface roughness, regardless of the type of wear. The (FV+L) group had the lowest volume loss, regardless of the type of wear performed: [(FV+L)+(E)] = 7.5%, [(FV+L)+(A)=7.3%, and [(FV+L)+(E+A)]=8.1%. The subgroup [(WPT)+(E+A)] had the highest volume loss (52.3%). The proposed treatments, therefore, were effective in controlling the increase in surface roughness. Additionally, laser irradiation showed satisfactory results for volume loss, notably when associated with the application of fluoride varnish beforehand.

KEYWORDS: Dental erosion. Fluoride varnish. Dental abrasion. Prevention. YSGG laser**.**

5 INTRODUCTION

The increase in life expectancy and the improvement in preventive techniques offered in the field of dentistry have contributed to a greater longevity and maintenance of natural teeth in the oral cavity [1]. However, the number of non-carious cervical lesions (NCCLs) gradually has increased due to behavioral changes and habits of the population [2,3].

These lesions arise due to a loss of structure at the cement-enamel junction without bacterial involvement, and are characterized by an irreversible loss of enamel and dentin [4,5]. Through a multifactorial etiology, the root surface associated with dental erosion, abrasion, abfraction, and attrition is exposed [6-8]. Periodontal treatments, mechanical injuries such as brushing, surgical treatments, increased consumption of acidic foods, and gingival recession are some of the determining factors for the development of NCCLs [6,9,10]. In addition, the combination of potential etiological factors for NCCL development, such as dental erosion and abrasion, can cause cervical dentin hypersensitivity and an accumulation of biofilm in the region [11].

Dental erosion can be endogenous or exogenous in origin [12,13]. The consumption of citrus fruits and juices, soft drinks, alcohol, vitamin C tablets, and isotonic drinks, because of their low pH and high erosive potential, are recognized in the literature as a source of injury to the dental structure, characterized by extrinsic dental erosion. Treatment is complex, due to a constant exposure to acidic challenges [3,14,15]. Additionally, the intrinsic factor of a change in pH can be stimulated by the hydrochloric acid that is present in the stomach reaching the oral cavity through vomiting, regurgitation, or gastroesophageal reflux, due to bulimia, anorexia, or a hiatal hernia [3,16].

The influence of toothbrushing, which is considered an abrasive event, on the progression of NCCLs is controversial. According to Shellis and Addy [17], under normal and adequate conditions, toothbrushing using toothpaste causes minimal dentin wear throughout life. However, the force applied during brushing can aggravate the progression of wear on the structures [5,6].

Preventive treatments for dental erosion are directed against causal factors and may include interventional changes to the diet and a reduced consumption of acidic drinks. Modifications can also be made to the tooth surface to increase its resistance to acidic challenges, for which fluorides have been effective [12,18]. Fluoride-containing solutions promote a protective effect, which has been demonstrated with *in vitro* and *in situ* studies [18-20]. The topical application of these fluoride agents, such as sodium fluoride (NaF), can protect against dental erosion through the formation of a CaF_2 layer on the surface [19].

Irradiation with the Er,Cr:YSGG laser on the dental surface causes an increase in the surface temperature and alters its chemical structure, leaving the surface less soluble [21]. For the prevention of abrasion, a previous study [22] has investigated the effectiveness of Er:YAG laser irradiation, with or without a fluoride agent, against abrasive wear on tooth enamel. The results showed that the proposed treatment was effective only when associated with the fluoride agent, since it promoted morphological changes in the enamel structure, which provided greater CaF_2 retention and helped to control abrasive wear. The behavior was expected to be similar with dentin.

Thus, the analysis of superficial roughness and 3D profilometry by means of laser scanning confocal microscopy are the parameters most commonly used to quantify and describe the surface alterations of dental substrates [23], since the presence of irregularities causes a greater accumulation of biofilm, increasing the chances of developing carious lesions and periodontal problems [24].

The null hypothesis of the present study was that the erosive and/or abrasive challenges and the different preventive treatments would not result in statistically significant differences in surface roughness and loss of volume in bovine root dentin.

6 OBJECTIVE

The aim of this *in vitro* study was to evaluate the influence of the Er,Cr:YSGG laser associated or not with 5% fluoride varnish on the surface roughness and volume loss of bovine root dentin subjected to erosive and/or abrasive wear, measured using confocal laser scanning microscopy.

7 MATERIALS AND METHODS

7.1 Selection of teeth

After approval by the Animal Experimentation Ethics Committee of the University of Uberaba under protocol number 028/2018, 60 bovine incisors without any cracks or wear were selected. The teeth were cleaned and then immersed in a 10%

formalin solution for disinfection (pH = 7) for 7 days. Afterwards, the teeth were washed and stored in distilled and deionized water at a temperature of 4ºC, which was changed daily for a period of 14 days [23].

7.2 Preparation of specimens

The incisors were sectioned by separating the coronary portion of the root using a diamond disk under refrigeration in the ISOMET 1000® cutting machine (Precision Saw Buehler, Illinois - USA). The first cut was made 1 mm below the enamel-cement joint. The second cut was made in the vestibulo-lingual direction, resulting in two halves (mesial and distal). Each half was again sectioned to obtain specimens with the dimensions 4.25 mm x 4.25 mm x 2.5 mm. The sides of the specimens were sanded with an Arotec APL-4 polishing machine (Series 41042, Arotec S.A. industry and commerce) under water cooling using a #600 sandpaper, resulting in a surface area of 18 mm^2. No polishing of the external (vestibular) surface of the specimen was performed. A 10% positive or negative variation in dimensions was allowed. Half of the surface of each specimen was covered with electrical tape. Two layers of red nail polish (Risqué Maybelline Ltda, Sao Paulo, SP, Brazil) and sculpting wax (Kota Industria, Cotia, Sao Paulo, Brazil) were applied, isolating the area. After this procedure, the electrical tape was removed, and each specimen had a free surface coated with the enamel and wax protection. The specimens were stored in distilled and deionized water at a temperature of 4º C until the proposed treatment was performed [23,25], at which point they were randomly divided into 12 subgroups (n = 10), according to the preventive treatments.

7.3 Experimental design

One hundred and twenty bovine root dentin specimens were made and randomly divided according to preventive treatments: G1 = without preventive treatment (WPT), G2 = 5% fluoride varnish (FV); G3 = irradiation with an Er,Cr:YSGG (L) laser and G4 = 5% fluoride varnish combined with the Er,Cr:YSGG laser (FV + L). The specimens were subdivided into 3 wear conditions (n = 10), which were: 1 = erosion (E); 2 = abrasion (A); and 3 = erosion followed by abrasion (E + A), equaling 12 subgroups. The quantitative variables were: surface roughness (parameter Ra in μm^2) and the percentage of volume loss.

7.4 Preventive treatment of specimens

The fluoride varnish (5% sodium fluoride) used was Duraphat® - 22600 ppm fluoride (Colgate Palmolive Ind. e Com. Ltda, São Paulo, SP, Brazil). A disposable applicator (KG Brush, KG Sorensen, Cotia, Sao Paulo, Brazil) was used to apply the varnish on the vestibular face of the previously waterproofed specimens. After 4 minutes, the excess was removed with a sterile gauze. A Er,Cr:YSGG laser (Waterlase Millennium, Biolase Technologies Inc., San Clemente, USA) was used with 600 µm diameter fiber (tip model: ZipTip MZ6 3 mm), at a wavelength of 2.78 µm, with 10 seconds of irradiation in the scanning mode 1 mm away from the target tissue, at 0.5 W power, a repetition rate of 5.0 Hz, and an energy density of 6.25 J/cm^2. Water cooling was not used to avoid compromising the preventive treatment, since tissue ablation can occur in the presence of water [23].

7.5 Erosive challenge

After the treatments, the specimens were submitted to an erosive challenge using Coca-Cola® (Uberlandia Refrescos LTDA, Uberlandia, MG, Brazil) with a pH of 2.43 at 4ºC, according to Arantes et al. [23] and modified for the present study. Each subgroup was placed separately in a beaker with 50 mL of solution for 5 minutes, with a magnetic stirrer (model 221-1, ABC-LAB Produtos e Equipamentos Laboratorios, Sao Bernardo do Campo, SP, Brazil). The erosive solution was then discarded, the specimens were washed with distilled and deionized water for 10 seconds, stored in artificial saliva, and placed in an oven at 37ºC between cycles. This procedure was performed twice a day, with a 2-hour interval between challenges, for a period of 10 days.

7.6 Abrasive challenge

Abrasive wear was performed on specimens in the pre-established subgroups, with brushing tests according to Cury et al. [26] and modified for the present study. Brushing was performed on the buccal surface of the specimens 1 hour after the second daily erosive challenge [24] and monitored by the same operator. The specimens were brushed *in vitro* with soft-bristled electric toothbrushes with rounded tips (Oral-B Professional Care 5000, Procter and Gamble, Marktheidenfeld, Germany), which simulated an oscillatory brushing technique in the direction parallel to the central marking that delineated the area that was subjected to the cycles. The dentifrice used was Colgate Total 12 Clean Mint (Colgate-Palmolive Industrial Ltda, Brazil). A solution (slurry) was obtained by mixing the toothpaste and distilled water at a proportion of 1:2 by weight. This solution was prepared 20 minutes before use. During the abrasive challenge, the electric toothbrush was attached to a device. The brush head (Precision Clean, Procter and Gamble) had three sets of soft bristles with different shapes and were positioned at different angles and heights. During brushing, the bristles touched with the dentin surface with a force of 2.0 N (≈200g) for 1 minute (166 oscillations/s), and every 15 seconds the slurry solution was injected between the bristles [26]. After each brushing, the specimens were washed with distilled and deionized water for 10 seconds, lightly dried with absorbent paper, and then kept in 25 mL of artificial saliva and stored at 37º C.

7.7 Analysis of surface roughness

After treatment followed by erosive and/or abrasive challenges, the specimens were analyzed using a laser scanning confocal microscope. The nail and wax (control area) of each specimen were removed using the Lecron instrument (Duflex Instruments, Juiz de Fora, MG, Brazil) prior to analysis by laser scanning confocal microscopy. There was no contact of the instrument with the central surface of the specimen, only on the sides.

The specimens were immersed in distilled and deionized water, inserted in an ultrasonic vat (Ultrasonic Cleaner 740D - Odontobrás - Ribeirao Preto, SP, Brazil), stirred for 5 minutes, and later positioned parallel to the table of the LEXT laser-scanning microscope (Olympus, Japan) with the aid of a parallelometer.

After selecting the central region of the 1 mm x 1 mm specimen, images were acquired using a 10x magnification lens (Fig 1). After obtaining the images, they were analyzed for surface roughness (parameter Ra in μm^2) and volume loss (%), and the control and experimental regions were compared. The data were acquired using specific software (OLS4000® - Olympus, Japan).

7.8 Analysis of the percentage of volume loss (3D profilometry)

The difference in the volume of the control and experimental regions of each specimen was determined, taking into account the midline of the graph. The data were obtained in μm^3 and, for statistical calculations, transformed into a percentage of lost volume.

7.9 Statistical analysis

Initially, the data were analyzed for distribution (Kolmogorov-Smirnov) and homogeneity (Levene). When these conditions were satisfied, the surface roughness data were subjected to one-way ANOVA and to Tukey's post-hoc for multiple comparisons. To analyze volume loss, the data were subjected to the Kruskal-Wallis test and the Dunn post-hoc. All statistical tests adopted a significance level of 5% ($\alpha = 0.05$).

8 RESULTS

8.1 Surface roughness

The surface roughness values are described in Table 1 (n = 10). In the control areas, all subgroups showed lower values of surface roughness that were statistically similar to each other ($p > 0.05$), and statistically different from the experimental areas ($p < 0.05$).

For the experimental areas, the surface roughness in the negative control subgroup [(WPT) + (E + A)] was the highest ($5.712\ \mu m^2 \pm 0.163\ \mu m^2$), with a statistically significant difference from the other subgroups ($p < 0.05$). There were no statistically significant differences between the L and (FV + L) subgroups, regardless of the type of wear ($p > 0.05$). Among the proposed treatments, the [(FV + (E + A)] subgroup had a surface roughness that was $3.355\ \mu m^2 \pm 0.098\ \mu m^2$, which was statistically significantly different from the other FV treatments groups ($p < 0.05$).

8.2 Percentage of volume loss

Regarding the percentages of volume loss that are described in Table 2, it was observed that (FV + L) showed the lowest volume loss ($p < 0.05$) regardless of the wear performed: [(FV + L) + (E)] = 7.5%, [(FV + L) + (A)] = 7.3%, and [(FV + L) + (E + A)] = 8.1%. However, the greatest volume loss was observed in the [(WPT) + (E + A)] subgroup, at 57.2%. Microscopically, there were great differences in the coloration of the control and experimental regions (Fig. 2).

9 DISCUSSION

Dental erosion and abrasion processes are important mechanisms of wear on the dental structure. In view of the numerous existing methods for assessing the superficial morphology of hard dental tissues, confocal laser scanning microscopy offers high resolution images and allows better surface characterization, such as enamel prisms, dentinal tubules, and demineralization areas [25] since it does not generate damage to the specimen surface [27], unlike scanning electron microscopy. Thus, the present study was carried out to evaluate the superficial morphology of bovine root dentin submitted to erosive challenges using a cola-based soft-drink and to abrasive challenges, through tooth brushing tests. The control and experimental regions showed results with statistically significant differences in surface roughness and volume loss ($p < 0.05$). Therefore, the null hypothesis that exposure to erosive and/or abrasive challenges and to different treatments would not result in statistically significant differences was rejected.

The initial standardization of the specimens was established through the superficial roughness results in the control areas, which showed no significant differences between the groups ($p > 0.05$). This corroborates results from a previous study [23], and demonstrates that waterproofing the specimens was effective, since the values presented were much lower than those in the experimental region ($p < 0.05$).

The erosive and/or abrasive challenges were carried out in order to simulate the oral conditions to which patients are subjected, either through the ingestion of beverages with an acidic pH or through toothbrushing [5,14].

NCCLs have been noted to be highly prevalent among the population [2.28]. According to some authors, their etiology is multifactorial, meaning they begin and progress due to the interaction of different wear processes [29-31], including erosion (corrosive wear), abrasion (abrasive wear), friction (occlusal overload, usually associated with abrasion), and attrition.

In advanced stages, NCCLs compromise the function and aesthetics of the teeth involved, and dentin hypersensitivity may appear, which often requires extensive restorative treatment [32].

For this study, the immersion of the specimens in a cola-based soft-drink (Coca-Cola®) was chosen due to its great erosive potential, widely discussed in the scientific literature [14,15,33], in addition to being consumed worldwide. Its pH is around 2.43 at the temperature at which it is consumed, which is below the critical dentin dissolution pH

(< 6.5) and promotes demineralization of the dentin surface [15]. This corroborates the current study results, making it possible to verify the superficial demineralization of dentin with Coca-Cola® in all erosion groups.

The data obtained for surface roughness demonstrated that there was a statistically significant difference in the control region compared to the experimental region ($p < 0.05$), for all subgroups. Subgroups L and (FV + L) showed that there was no statistical difference between the treatments performed ($p > 0.05$). An increase in dentin roughness, as observed in the present study, can lead to greater bacterial adhesion to the surface and greater accumulation of biofilm, making the dental substrate more susceptible to external stains [34].

The subgroup [(WPT) + (E + A)] showed the highest surface roughness (5.712 μm^2) and volume loss (57.2%), demonstrating the potential for dentin wear caused by two combined challenges, that is, erosion followed by abrasion, which reinforces the need for preventive measures.

NaF can provide protection to tooth enamel by releasing calcium ions into oral fluids in acidic conditions, which can increase the concentration of calcium in saliva. The degree of saturation of enamel hydroxyapatite can thus change, contributing to the inhibition of demineralization and an increase in remineralization [21,35]. Considering that hydroxyapatite is one of the main constituents of enamel and dentin, it is expected that fluoride protect dentin against the demineralization process through a similar mechanism [23]. Kim et al. [36] demonstrated that during the demineralization and remineralization processes, fluoride agents promote the formation of fluoroapatite or fluoride hydroxyapatite, which are more resistant to dissolution in an acid medium.

In the present study, the FV group with the highest surface roughness was the [(FV) + (E + A)] subgroup, at 3.355 μm^2. This was statistically different from the other FV-treated specimens. However, it presented a lower volume loss (23.9%) for erosion than the other types of wear. This can be explained by the fact that fluoride, even in the form of varnish, is easily removed with brushing or in an acidic medium [37].

According to a previous study, dentin wear is related to toothpaste abrasiveness, measured using Relative Dentine Abrasivity (RDA). The International Organization for Standardization (ISO # 11609) recommends that dentifrices be exposed to an RDA less than 250 [17]. It is important to note that the Colgate Total 12 Clean Mint toothpaste used in the present study has 1450 ppm of fluoride, is used daily, and has silica as an abrasive agent (RDA = 139.1 ± 4.51) [38], which may have helped to remove the fluoride varnish. This can be seen by comparing the volume loss values of the subgroups [(FV) + (A)] = 34.7% and [(WPT) + (A)] = 35.1%, which were statistically similar ($p > 0.05$), corroborating the findings of another study [38] that evaluated the same toothpaste, showing RDA higher than Sensodyne Protection and Repair (102.6 ± 3.24) and Colgate Sensitive Pro-Relief (81 ± 3.55).

Therefore, it is suggested that preventive treatment with a single application of the fluoride agent seems to have influenced the surface roughness of dentin subjected to erosion and abrasion, likely promoting the formation of a CaF_2 layer [19], which may have been removed by tooth brushing. The abrasiveness of the toothpaste and the low pH of the beverage used can negatively influence surface roughness, especially since these events are associated in the oral cavity.

Regarding the electric brush used, the specimens were brushed with oscillating rotation movements, with a brush head with three sets of soft bristles of different shapes positioned at different angles [39]. Dentin wear occurred due to the greater number of brushing movements produced by the electric method together with the application of 2 N of force. The action of the bristles associated with the high abrasiveness of the toothpaste promoted greater dentin wear [40-42].

In previous studies [3,43], NaF has been shown to have excellent results in reducing the progression of dental erosion of enamel and protecting the precipitation of calcium fluoride on eroded dental surfaces, especially when used in high concentrations [43, 44]. In the present study, fluoride varnish proved to be more effective in preventing erosion than the WPT group. However, for situations of abrasion or (E + A), it did not have the same protective effect.

For volume loss, there was no statistically significant difference between the groups irradiated with L ($p > 0.05$). The (FV + L) group presented the lowest values of volume loss, regardless of the type of wear performed. The combination of laser and fluorides has been shown to potentiate each other, improving the incorporation of fluoride ions into the root dentin and increasing its resistance to acid [9,23,25,45].

The dental surgeon has a fundamental role in guiding patients in the importance of a less acidic diet, the use of toothbrushes with soft bristles, and less abrasive toothpaste in order to prevent the onset and progression of NCCLs.

For future studies, it is important to analyze other physical-mechanical properties, such as the color stability of the dental substrate, the longitudinal microhardness, and the bond strength of the substrate to the dental materials in order to investigate the longevity of the materials and the restorative techniques used in irradiated dentin.

10 CONCLUSION

Considering the results obtained and the limitations of this *in vitro* study, the proposed treatments were determined to be effective in controlling an increase in surface roughness. For volume loss, laser irradiation showed satisfactory results, notably when associated with the application of fluoride varnish beforehand.

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12 CONFLICT OF INTEREST

The authors state that there are no conflict of interest.

13 ROLE OF FUNDING SOURCE

CAPES (PROSUP- BOLSA), CNPq (PIBIC) and FAPEMIG (PIBIC) provided scholarships and PAPE-UNIBE made possible the financial vialization of this project.

14 ETHICAL APPROVAL

This project followed all the ethical principles for medical research, according to Declaration of Helsinki.

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LEGENDS

Fig 1. Representative image of the surface roughness analysis. To the left of the green line is the control region and to the right the experimental region. For the statistical analysis, the values of the Ra parameter of roughness were used.

Fig 2. Representative images of specimens analyzed in confocal microscopy from laser scanning. The blue arrow represents the control region and the red arrow represents the experimental region.

TABLES

Table 1. Mean values and standard deviation of the surface roughness of the groups (μm²), considering the control and experimental regions.

PREVENTIVE TREATMENT	*TYPE OF CHALLENGE*	*SUBGROUP CODE*	*CONTROL REGION*	*EXPERIMENTAL REGION*
G1 – Without Preventive Treatment (WPT)	1.Erosion	[(WPT)+(E)]	0.812 (0.061)[a]	3.592 (0.092)[c]
	2.Abrasion	[(WPT) +(A)]	0.867 (0.084)[a]	3.734 (0.075)[c]
	3.Erosion + Abrasion	[(WPT) + (E+A)]	0.845 (0.077)[a]	5.712 (0.163)[d]
G2- 5% Fluoride Varnish (FV)	1.Erosion	[(FV) + (E)]	0.844 (0.069)[a]	2.132 (0.084)[b]
	2.Abrasion	[(FV) + (A)]	0.832 (0.057)[a]	2.251 (0.090)[b]
	3.Erosion+ Abrasion	[(FV) + (E+A)]	0.872 (0.080)[a]	3.355 (0.098)[c]
G3- Er,Cr:YSGG Laser (L)	1.Erosion	[(L) + (E)]	0.846 (0.056)[a]	2.101 (0.059)[b]
	2.Abrasion	[(L) + (A)]	0.837 (0.081)[a]	2.136 (0.080)[b]
	3.Erosion + Abrasion	[(L)+(E+A)]	0.866 (0.085)[a]	2.205 (0.084)[b]
G4- Varnish + Laser (FV+L)	1.Erosion	[(FV+L) + (E)]	0.841 (0.074)[a]	2.015 (0.087)[b]
	2.Abrasion	[(FV+L) + (A)]	0.840 (0.053)[a]	2.041 (0.095)[b]
	3.Erosion + Abrasion	[(FV+L) + (E+A)]	0.849 (0.083)[a]	2.263 (0.086)[b]

* Equal letters represent statistical similarity between groups (p>0.05)

Table 2. Mean values and standard deviations (SD) of volume loss (%) considering the control and experimental regions.

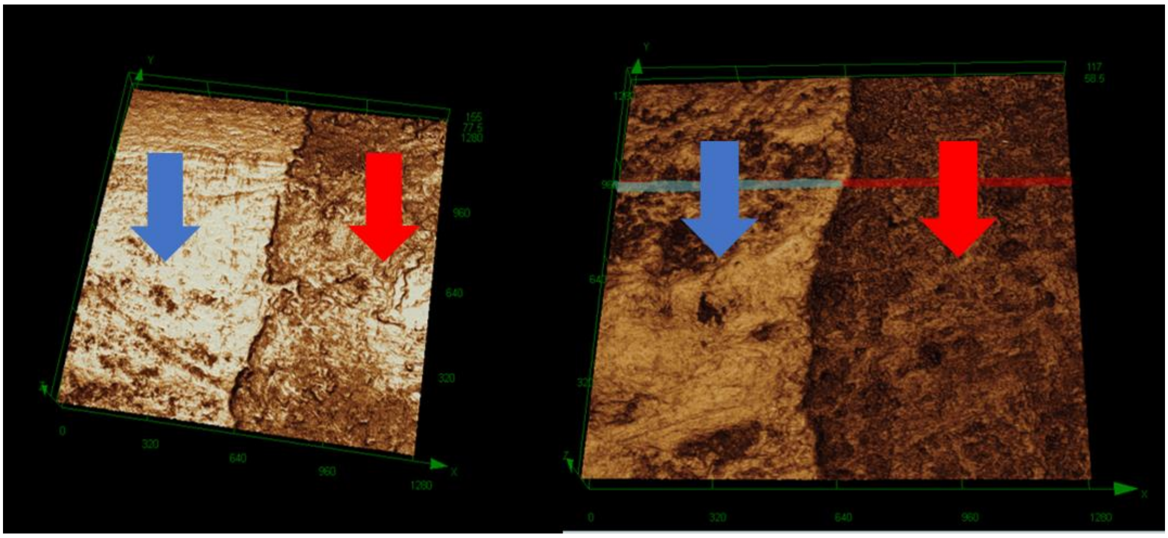
PREVENTIVE TREATMENT	*TYPE OF CHALLENGE*	*SUBGROUP CODE*	*VOLUME LOSS % (SD)*
G1 - Without preventive treatment (WPT)	1.Erosion	[(WPT)+(E)]	37.8 (2.9)[d]
	2.Abrasion	[(WPT) +(A)]	35.1 (3.5)[d]
	3.Erosion + Abrasion	[(WPT) + (E+A)]	57.2 (4.1)[e]
G2- 5% Fluoride Varnish (FV)	1.Erosion	[(FV) + (E)]	23.9 (2.4)[c]
	2.Abrasion	[(FV) + (A)]	34.7 (3.0)[d]
	3.Erosion+ Abrasion	[(FV) + (E+A)]	36.7 (3.9)[d]
G3- Er,Cr:YSGG Laser (L)	1.Erosion	[(L) + (E)]	13.4 (2.6)[b]
	2.Abrasion	[(L) + (A)]	14.0 (3.1)[b]
	3.Erosion + Abrasion	[(L)+(E+A)]	14.4 (2.3)[b]
G4- Varnish + Laser (FV+L)	1.Erosion	[(FV+L) + (E)]	7.5 (1.2)[a]
	2.Abrasion	[(FV+L) + (A)]	7.3 (1.5)[a]
	3.Erosion + Abrasion	[(FV+L) + (E+A)]	8.1 (1.7)[a]

* Equal letters represent statistical similarity between groups ($p>0.05$)

FIGURES

Fig 1

Fig 2



16. CONCLUSÃO

16 CONCLUSÃO

Diante dos resultados obtidos e com as limitações de um estudo *in vitro*, conclui-se que os tratamentos propostos foram eficazes no controle do aumento da rugosidade superficial. Para a perda de volume, a irradiação laser apresentou resultados satisfatórios, notadamente quando associada à aplicação prévia de verniz fluoretado.

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APÊNDICES

APÊNDICES

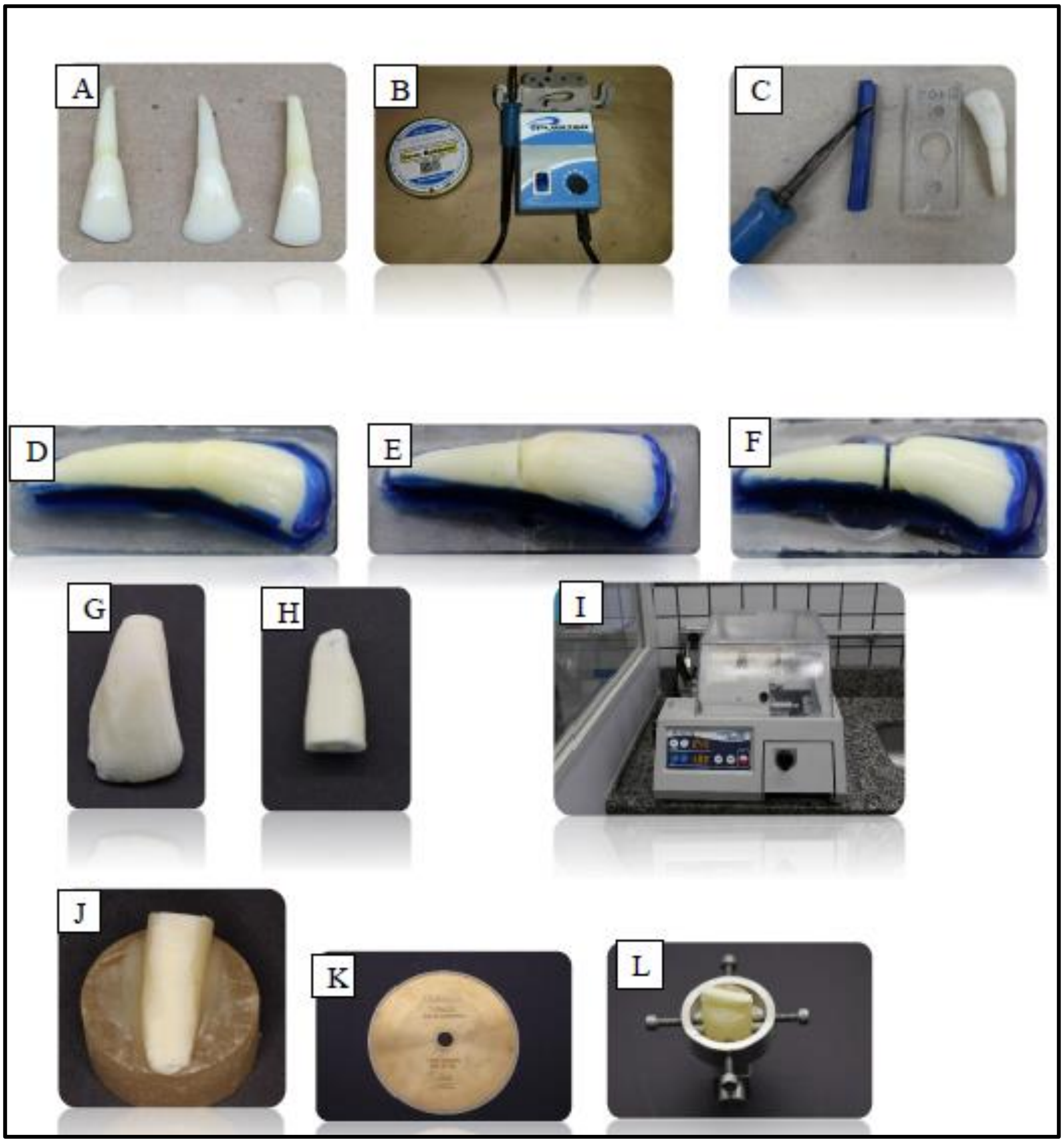

Figura 1. Preparo dos espécimes. A- Dentes hígidos bovinos. B- Gotejador elétrico. C- Gotejador elétrico e cera para fixação do dente na placa acrílica. D- Dente fixado com cera na placa acrílica. E- Início de separação coroa e raiz. F- Separação coroa e raiz. G- Coroa bovina. H- Raiz bovina. I- Máquina de corte - ISOMET® 1000. J- Cilindro acrílico e fixação de raiz. K- Disco diamantado. L- Dispositivo utilizado para o preparo dos espécimes.

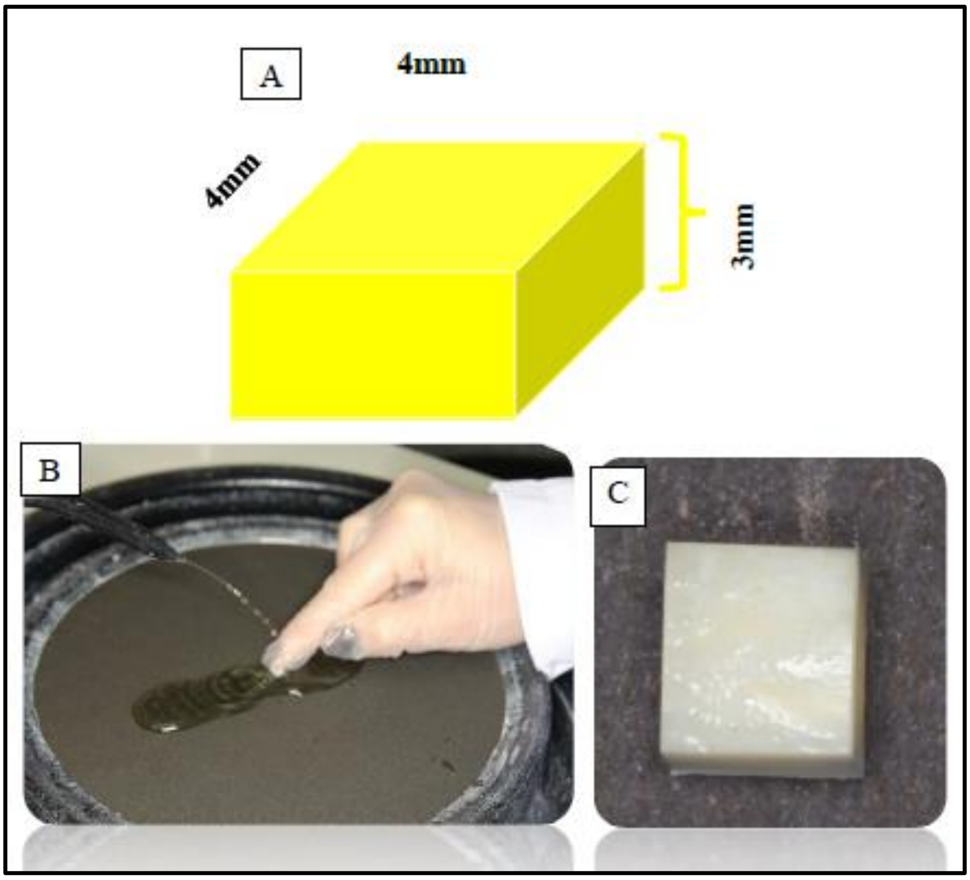

Figura 2: Padronização dos espécimes. A- Dimensão preconizada. B- Lixadeira e Politriz Metalográfica APL (Arotec). C- Espécime Padronizado.

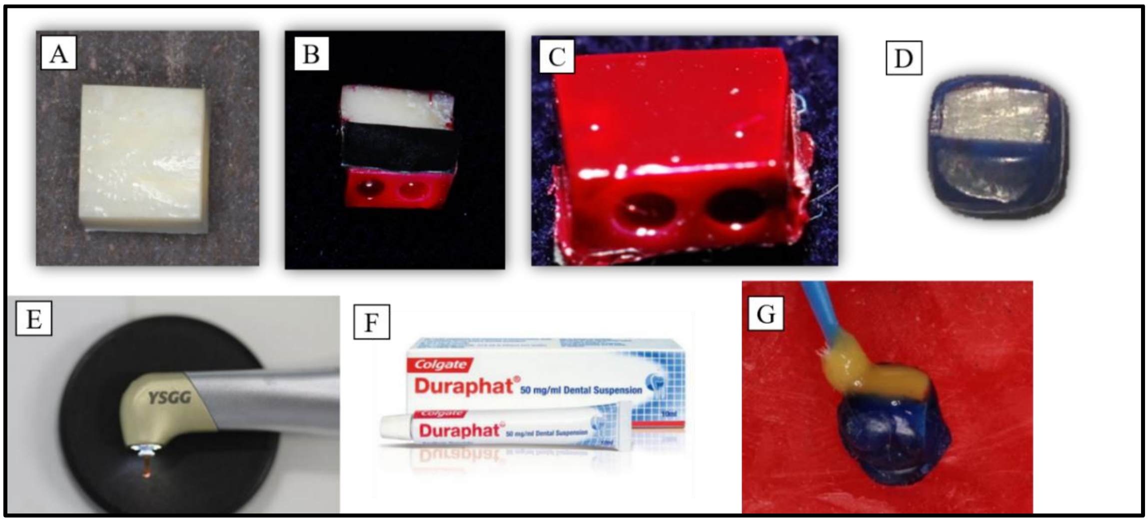

Figura 3. Tratamento preventivo dos espécimes. A- Espécime padronizado. B- Espécime dividido em região controle e experimental (fita isolante). C- Espécime com as camadas de esmalte de unha. D- Espécime pronto para receber o tratamento. E- Laser Er,Cr:YSGG. F- Verniz fluoretado 5%. G- Aplicação do verniz no espécime com microbrush.

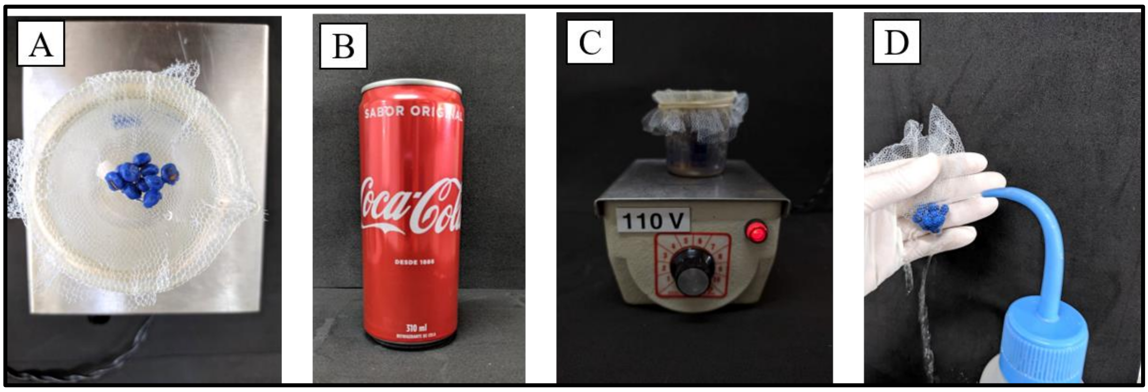

Figura 4. Desafio erosivo. A- Espécimes dispostos para imersão em solução Coca-Cola. B- Solução utilizada. C- Agitação da solução em agitador magnético. D- Lavagem dos espécimes após os 5 minutos de imersão.

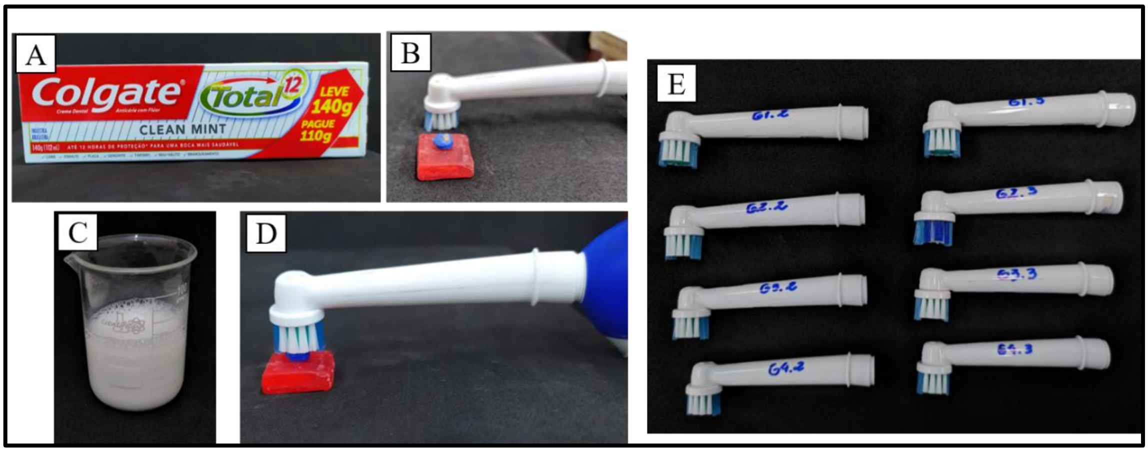

Figura 5. Desafio abrasivo. A- Dentifrício utilizado. B- Espécime posicionado. C- Solução *slurry*. D- Escovação do espécime. E- Escovas identificadas de acordo com cada grupo.

ANEXOS

ANEXOS

Anexo 1: Carta de aprovação do Comitê de Ética em Experimentação Animal da Universidade de Uberaba

UNIUBE Educação e Responsabilidade Social — Comitê de Ética em Experimentação Animal

Ofício CEEA-043/2019 — Uberaba, 31 de maio de 2019.

CERTIFICADO

Certificamos que o protocolo nº 028/2018 relativo ao projeto intitulado *"Influência do laser ER, CR: YSSG associado ou não ao verniz fluoretado 5% na rugosidade superficial e no perfil de desgaste da dentina radicular bovina submetida a desafios erosivos e/ou abrasivos"* que tem como responsável **Prof. César Penzazzo Lepri,** está de acordo com os Princípios Éticos da Experimentação Animal, adotados pelo Comitê de Ética em Experimentação Animal (CEEA/UNIUBE) regido pela lei nº 11.794/08.

CERTIFICATE

We hereby certify that the protocol nº 028/2018 related to the project entitled ***"Influence of Er,Cr:YSSG laser, associated or not to 5% fluoride varnish, on surface roughness and wear profile of bovine root dentin submitted to erosive and/or abrasive challenges"***, under the supervision of Prof. César Penzazzo Lepri, is in agreement with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation (CEEA/UNIUBE) according to the law nº 11.794/08.

Atenciosamente,

Profa. Joely Ferreira Figueiredo Bittar
Coordenadora do CEEA-UNIUBE

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Anexo 2: Normas para publicação no periódico Lasers in Medical Science

- Primeira Página

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Downloads 209,23 (2018)

Aims and scope

Lasers in Medical Science (LIMS) has established itself as the leading international journal in the rapidly expanding field of medical and dental applications of lasers and light. It provides a forum for the publication of papers on the technical, experimental, and clinical aspects of the use of medical lasers, including lasers in surgery, endoscopy, angioplasty, hyperthermia of tumors, and photodynamic therapy. In addition to medical laser applications, LIMS presents high-quality manuscripts on a wide range of dental topics, including aesthetic dentistry, endodontics, orthodontics, and prosthodontics.

The journal publishes articles on the medical and dental applications of novel laser technologies, light delivery systems, sensors to monitor laser effects, basic laser-tissue interactions, and the modeling of laser-tissue interactions. Beyond laser applications, LIMS features articles relating to the use of non-laser light-tissue interactions.

Instructions for Authors

Types of papers

Original Article – limited to 4000 words, 45 references, no more than 5 figures

Review Article – limited to 5000 words, 50 references, no more than 5 figures

Brief Report - limited to 2000 words, 25 references, no more than 4 figures - Case Reports will not be accepted!

Letter to the Editor – up to 600 words

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Manuscript Submission

- Última Página

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Anexo 3. Comprovante de submissão do artigo

Lasers in Medical Science - Submission Notification to co-author

Você encaminhou esta mensagem em Seg, 03/02/2020 16:59

Lasers in Medical Science <em@editorialmanager.com>
Seg, 03/02/2020 12:51
Você

Re: "Influence of the Er,Cr:YSGG laser, with or without a 5% fluoride varnish, as preventive treatment of bovine root dentin submitted to erosive and/or abrasive challenges"
Full author list: Gabriella Rodovalho Paiva, DDS; Regina Guenka Palma-Dibb, PhD; Juliana Jendiroba Faraoni, PhD; Maria Angelica Hueb de Menezes Oliveira, PhD; Denise Tornavoi de Castro, PhD; Vinicius Rangel Geraldo-Martins, PhD; Cesar Penazzo Lepri, Adjunct Professor

Dear DDS Gabriella Paiva,

We have received the submission entitled: "Influence of the Er,Cr:YSGG laser, with or without a 5% fluoride varnish, as preventive treatment of bovine root dentin submitted to erosive and/or abrasive challenges" for possible publication in Lasers in Medical Science, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. PhD Cesar Penazzo Lepri who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office
Lasers in Medical Science

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