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GUILHERME GONÇALVES DA CRUZ

EFEITO DA INSTRUMENTAÇÃO PERIODONTAL NA ESTRUTURA DENTÁRIA AFETADA PELA RADIAÇÃO IONIZANTE: ESTUDO IN VITRO

Effect of periodontal instrumentation on tooth structure affected by ionizing radiation: in vitro study

Dissertação apresentada à Faculdade de Odontologia da Universidade de Uberlândia, para obtenção do Título de Mestre em Odontologia na Área de Clínica Odontológica Integrada.

Uberlândia, 2022

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SUMÁRIO

RESUMO

Avaliar a importância da terapia periodontal não cirúrgica em pacientes radioterápicos na região de cabeça e pescoço. Os efeitos que diferentes protocolos de raspagem têm na morfologia e rugosidade do substrato afetado pela radiação ionizante. Cento e trinta incisivos bovinos extraídos foram divididos aleatoriamente em dois grupos (n=65): não irradiados (NIR) e irradiados (IR). Eles foram alocados aleatoriamente de acordo com o tipo de protocolo de intervenção: NIT: sem instrumentação; NIT/PP: profilaxia com pasta profilática fina com taça de borracha acoplado a dispositivo de rotação de baixa velocidade sob irrigação constante por 15 segundos; HS: instrumentação manual com 15 movimentos apical-coronais; HS/PP: instrumentação manual seguida de protocolo de profilaxia; US: instrumentação ultrassônica, com 15 ciclos apical-coronais. US/PP: instrumentação ultrassônica seguida de protocolo de profilaxia. A rugosidade da superfície radicular foi medida com perfilômetro e as características morfológicas das superfícies radiculares foram analisadas por microscopia eletrônica de varredura (MEV). Os dados obtidos do perfilômetro foram analisados utilizando ANOVA two-way seguida de Tuckey (α= 0,05). Para MEV foi utilizado o teste Kappa para avaliação do interobservador e regressão logística ordinal para analisar o efeito de cada fator nos escores. Para os protocolos com PP, os valores de Ra e Rz após os tipos de instrumentação foram menores independente da radioterapia. Os grupos US tiveram os maiores valores de Ra e Rz independente da radioterapia. Para a variação Ra, foram encontrados os seguintes valores: para os grupos NIr (HS-0,31 $\pm$ 0,05, US-1,51 $\pm$ 0,23) apresentaram os valores mais aumentados, a profilaxia tem efeito apenas no protocolo (US/PP-0,19 $\pm$ 0,06); Para Ir (HS-0,46 $\pm$ 0,07, US-2,23 $\pm$ 0,25) também apresentou os valores mais pronunciados, quando comparado ao grupo Nir os valores apresentaram diferenças estatísticas, a profilaxia tem efeito em ambos os protocolos (HS/PP-0,13 $\pm$ 0,04 , US /PP-0,20 $\pm$ 0,03,). As diferenças estatísticas entre os valores encontrados para RZ seguiram os parâmetros de Ra. Para MEV foi observada uma concordância moderada entre os avaliadores (Kappa global = 0,443). O substrato não afetou os escores. Por outro lado, para a regressão logística ordinal, tanto a instrumentação manual quanto a ultrassônica resultaram em pontuações mais altas do que nenhuma instrumentação e a profilaxia reduziu os escores. Tanto a instrumentação manual quanto a ultrassônica resultaram em superfície radicular irradiada mais rugosa do que a dentina não irradiada. No entanto, o procedimento de profilaxia após a instrumentação reduziu significativamente a rugosidade das superfícies radiculares instrumentadas com curetas manuais ou raspadores ultrassônicos. A profilaxia sozinha não alterou significativamente a rugosidade da superfície.

Palavras-chave: Radioterapia, terapia periodontal, radiação ionizante

ABSTRACT

Discuss the importance of non-surgical periodontal therapy in patients undergoing radiotherapy in the head and neck region. The effects that different scaling protocols have on the morphology and roughness of the substrate affected by ionizing radiation. One hundred and thirty extracted bovine incisors were randomly divided into two group (n=65): non-irradiated (NIR) and irradiated (IR). They were randomly subdivided according to the type of protocol of intervention: NIT: no instrumentation; NIT/PP: only prophylaxis with a fine prophylactic paste using a rubber cup coupled with a low-speed rotation device under constant irrigation for 15 seconds; HS: scaling using hand instrumentation with 15 apical-coronal movements; HS/PP: Hand instrumentation followed by prophylaxis protocol; US: scaling using a ultrasonic instrumentation, with 15 apical-coronal cycles. US/PP: ultrasonic instrumentation followed by prophylaxis protocol. The root surface roughness was measured with a profilometer and the morphological features of the root surfaces were analyzed using SEM. The data obtained from profilometer for were analyzed using, two-way ANOVA followed Tuckey`s (α= 0,05). For SEM Kappa test was used for assessing the inter-observer and ordinal logistic regression was used to analyze the effect of each factor on the scores. For the protocols with PP, the Ra and Rz values after the types of instrumentation were lower regardless of radiotherapy. The US groups had the highest Ra and Rz values regardless of radiotherapy. For the Ra variation, the following values were found: For groups NIr (HS-0.31 ±0.05, US-1.51 ±0.23) presented the most pronounced values, the prophylaxis has effect only on protocol (US/PP-0.19 ±0.06) ; For Ir (HS-0.46 ±0.07, US-2.23 ±0.25) also presented the most pronounced values, when compared to the Nir group the values presented statistical differences, prophylaxis has effect in both protocols (HS/PP-0.13 ±0.04, US/PP-0.20 ±0.03,). The statistical differences between the values found for RZ followed the parameters of Ra. For SEM a moderate concordance among the evaluators was observed (overall Kappa = 0.443). The substrate did not affect the scores. On the other hand, for ordinal logistic regression, both manual and ultrasonic instrumentation resulted in higher scores than no instrumentation; and the prophylaxis reduced the scores. Both manual and ultrasonic instrumentation resulted in rougher root surface irradiated than non-radiated dentin. However, the prophylaxis procedure following the instrumentation significantly reduced the roughness of root surfaces instrumented with either hand curettes or ultrasonic scallers. Prophylaxis alone did not significantly change the surface roughness.

Key words: Radiotherapy, periodontal therapy, radiation ionizing

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

A radioterapia pode ser utilizada para o tratamento de neoplasias malignas podendo ser empregada como tratamento primário, coadjuvante à cirurgia ou em associação a quimioterapia (Buglione *et al*., 2016). Os efeitos citotóxicos da radiação ionizante atingem tanto células malignas quanto sadias, quando aplicada em região de cabeça e pescoço os tecidos adjacentes da cavidade oral também são afetados, podendo lesar a estrutura dentária e todo periodonto (Ammajan *et al*., 2013; Hancock *et al*., 2003; Irie *et al*., 2018).

As alterações orais agudas da radioterapia são: mucosite ou estomatite e as alterações crônicas: xerostomia, cárie de radiação, trismo, alterações no paladar, dermatite, necrose de tecidos moles e osteorradionecrose, e podem perdurar por anos (Sroussi *et al*., 2017; Baudelet *et al*., 2019). Essas alterações podem influenciar no grau de higienização do paciente oncológico, tornando-o suscetível à doença periodontal. Estes casos requerem uma abordagem multidisciplinar para reduzir a intensidade dos efeitos colaterais orais induzidos pela radiação ionizante, entender o papel do cirurgião dentista na prevenção e tratamento ainda gera dúvidas sobre qual conduta deve ser realizada (Irie *et al*., 2018; Yildirim *et al.,* 2021).

As defesas locais diminuídas e a salivação alterada favorecem a formação de novas bolsas periodontais, o que torna a atuação do periodontista na equipe oncológica essencial (Yildirim *et al.,* 2021). A terapia periodontal não cirúrgica é considerada a primeira abordagem para controle da infecção periodontal, com intuito de diminuir a carga bacteriana da bolsa periodontal e auxiliar na remoção de depósitos rígidos como o cálculo, que causam agravamento da infecção (Arora *et al*., 2016). Esta terapia pode ser executada utilizando vários métodos, que incluem instrumentos manuais, aparelhos sônicos, ultrassônicos, terapia a laser e do uso de microscópio operatório (Tsurumaki *et al.*, 2011; Yildirim *et al.* ,2021). O uso desses instrumentos tem demonstrado aumentar a rugosidade da superfície radicular devido à formação de irregularidades e sulcos durante a instrumentação, o que pode aumentar a adesão do biofilme no ambiente supragengival (Arora *et al.,* 2016). Para reduzir os nichos de acúmulo de biofilme, a instrumentação deve ser acompanhada de polimento da superfície para melhorar o controle mecânico do biofilme bucal (Yurdaguven *et al.,* 2012). No entanto essas alterações ocorrem em estrutura dental normal, os efeitos em estrutura alterada pela radioterapia ainda carecem de maiores evidências científicas.

2. PROPOSIÇÃO

Avaliar o efeito de diferentes métodos de instrumentação (instrumentação manual ou ultrassônica seguida ou não de profilaxia) na rugosidade superficial da dentina radicular de dentes bovinos hígidos ou irradiados.

3. CAPITULO 1

Effect of periodontal instrumentation on tooth structure affected by ionizing radiation: in vitro study

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Effect of periodontal instrumentation on tooth structure affected by ionizing radiation: in vitro study

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Abstract

Background: The non-surgical periodontal therapy in patients undergoing radiotherapy in the head and neck region can have impact on tooth structure. The aim of this study was to evaluate the effects of different scaling protocols on the morphology and roughness of the root dentin substrate influenced by ionizing radiation. **Material and Methods:** One hundred and thirty extracted bovine incisors were randomly divided into two sub-group: non-irradiated (NIR) and irradiated (IR). They were randomly subdivided according to the type of non-surgical periodontal protocol of intervention (n = 20): NIT: no instrumentation;

NIT/PP: prophylaxis with a fine prophylactic paste using a rubber cup coupled with a low-speed rotation device under constant irrigation for 15 seconds; HS: scaling using hand scaling with 15 apical-coronal instrument movements; HS/PP: Hand scaling followed by prophylaxis protocol; US: ultrasonic scaling with 15 apical-coronal cycles; US/PP: ultrasonic scaling followed by prophylaxis protocol. The root dentin surface roughness was measured with a profilometer (Ra/Rz - □m) and the morphological of the dentin surfaces were analyzed using scanning eclectic microscopy (SEM). The Ra data were analyzed using two-way ANOVA followed Tukey`s test (α= 0.05). The SEM data was analyzed by Kappa test assessing the inter-observer and ordinal logistic regression was used to analyze the effect of each factor on the scores. **Results:** The PP protocol had significant the Ra and Rz values after the types of scaling, regardless of radiotherapy. The US had significant higher Ra and Rz values regardless of radiotherapy. NIr presented the most pronounced Ra increasing values, and the prophylaxis has effect only when the US protocol was used. For Ir groups prophylaxis has effect in both protocols. SEM results showed a moderate concordance among the evaluators was observed (overall Kappa = 0.443). The score levels were not influenced by the substrate protocol. Ordinal logistic regression showed that both HS and US resulted in higher scores than NIT, irrespective of IR presence. **Conclusions:** The prophylaxis reduced the scores levels for all groups. Prophylaxis alone did not significantly change the Ra parameter of the dentin substrate irradiated or not.

Key words: Radiotherapy, periodontal therapy, radiation ionizing

Introduction

Head and neck cancers represent a heterogeneous group of malignant tumors involving up to 4% of overall cancers worldwide and causing approximately 360,000 deaths annually, of which 55,550 are in the oral cavity.[1] Factors such as the type of cancer, staging, and location define the type of treatment. Radiotherapy (RT) can be used as the primary treatment combined or not with surgery chemotherapies.[2,3] RT can affect adjacent tissues in the oral cavity when applied to the head and neck region since the ionizing radiation has cytotoxic effects and affects normal and malignant cells.[2,4] Therefore, damage to the tooth structure and the entire periodontium can be observed as an adverse effect of RT.[5-8]

Several secondary effects related to RT have been reported in the oral cavity. Mucositis, opportunistic infections, and thickened secretions to sensory disturbances are some acute oral alterations. [4,9] On the other hand, xerostomia, radiation caries, trismus, dysgeusia, dermatitis, soft tissue necrosis, and osteoradionecrosis are commonly observed chronic alterations.[9,10-14] Events such as hyperemia, inflammation, thrombosis, cell loss, hypo vascularization and fibrosis generate morphological/histological changes in the periodontium and are tailored to the exposure time to irradiation.[7,10-12]

All these changes can impair the patients who underwent RT to adequately control the oral biofilm increasing the risk of periodontal diseases.[7,12,13] Reduced local defenses and altered salivation favor the formation of periodontal pockets. Thus, a periodontist in the oncology team can be necessary to achieve satisfactory periodontal health.[13,14] Non-surgical periodontal therapy is the first approach to control periodontal infection and aims to reduce the bacterial content in the periodontal pocket and remove hard deposits such as calculus.[8,15,16] This initial therapy can be performed using hand instruments, ultrasonic scalers, rotatory instruments, laser therapy and an operating microscope.[16-19]

It has been demonstrated that using these instruments results in rougher root surfaces by increasing the irregularities and sulcus, which favors the biofilm adhesion.[15,18,20,21] Therefore, the periodontal instrumentation must be followed by surface polishing that improves the mechanical control of the biofilm by patients.[22,23] Usually, these surface changes are evaluated in sound tooth structure. However, the RT can affect the structure of both enamel (crystalline structure, acid solubility of enamel, microhardness) and dentin (elastic modulus, microhardness, matrix metalloproteinases).[11,24] The aim of this study was to evaluate the effect of different instrumentation methods (hand or ultrasonic instrumentation followed or not by prophylaxis) on the surface roughness of root dentin of sound or irradiated bovine teeth. The null hypothesis tested was that both the instrumentation method and tooth irradiation would not affect the roughness of root dentin.

Material and method

Sample separation and irradiation

One hundred and thirty extracted bovine incisors were selected for this study. The teeth were stored at 4ºC in distilled water that was replaced weekly. The crowns were separated from the roots at the enamel-cementum junction with a double-faced diamond disk (KG Sorensen, Barueri, SP, Brazil) used with a low-speed handpiece (KaVo do Brasil Ltda, Joinville, SC, Brazil) under copious water spray. An area of 5 x 5 mm^2 was delimited on the root surface and mounted individually in a rectangle (2,5 cm in length x 1,5 cm in width and 1,0 cm in high) polystyrene resin (Cristal, Piracicaba, SP, Brazil), using a polyether impression material (Impregum Soft; 3M ESPE, St. Paul, MN, USA) exposing the buccal surface. The surface to be analyzed was finished using 600-, 800-, 1200- and 2000-grit silicon-carbide papers (Norton, Campinas, SP, Brazil), and polished with metallographic diamond pastes (6, 3, 1, 1⁄4 µm; Arotec, São Paulo, SP, Brazil). The samples were washed with three 10-minutes ultrasound baths (Cristofoli, Campo Mourão, Paraná, Brazil) with absolute alcohol to remove debris.

Half of the samples were randomly selected to be irradiated, while the others were kept distillated water. During irradiation, the teeth were fixed in utility wax plates (Technew, Rio de Janeiro, RJ, Brazil) and immersed in distillated water at room temperature and completely covering the teeth. Every 15 days, the distillated water was discarded, and the specimens were stored in new distillated water. The irradiation protocol consisted of a total dose of 70 Gy, with 2 Gy daily applied 5 days a week, for 7 weeks with X-rays from a linear accelerator (Clinac 600C Varian®—Palo Alto, CA, USA, Beam 6 MV). The non-irradiated samples were stored in distilled water at 4 ◦C during the time required to complete the irradiation of other samples[29].

Instrumentation

Both, irradiated and non-irradiated samples, were randomly divided to receive one of the following instrumentations (n=20): Hand - scaling using hand instrumentation (Gracey curettes 5/6; Hu-Friedy, Chicago, Illinois, USA) with 15 apical-coronal movements; Ultrasonic: scaling using an ultrasonic instrumentation (Piezon PM200; EMS, Nyon, Switzerland), with 15 apical-coronal cycles; or no-instrumentation (control). The samples were fixed on a bench vise (185089, MTX, China) during the instrumentation/prophylaxis procedures. One operator (GGC) performed all scaling and polishing procedures. A second blinded operator (GC) evaluated the samples with profilometer (Surftest SJ-201P; Mitutoyo Corporation, Kawasaki, Kanagawa, Japan)

Determination of the Root Surface Roughness

The surface roughness was measured before and after the interventions. The root surface roughness was measured with a profilometer. Five parallel readings were performed on an area previously delimited, and the average was calculated. The readings were performed with a 0.25-mm cut-off and 1.25-mm measurement length at a speed of 1 mm/s, covering a distance of 3 mm. It was calculated both the mean of the recorded peaks and valleys (Ra) and mean roughness depth (Rz), which is the maximum distance between the highest peak and the deepest valley. The profilometer was positioned so that the height of the reading tip was adapted to the previous map of each sample.

Post-instrumentation prophylaxis

After the measurement of surface roughness, all samples were submitted to prophylaxis with a fine prophylactic paste (Herjos-F; Vigodent S.A., Rio de Janeiro, RJ, Brazil). A rubber cup coupled to a low-speed rotation device was used under constant irrigation for 15 seconds. The surface roughness was measured again as described previously, and five sample of each experimental condition was randomly selected to be analyzed under Scanning Electronic Microscopy (SEM)

Scanning Electronic Microscopy (SEM) Analysis

The morphological features of the root surfaces were analyzed using SEM. Five samples from each experimental condition (irradiation vs. intervention) were randomly selected. These samples were cleaned in an ultrasonic bath (Cristofoli, Campo Mourão, Paraná, Brazil) with distilled water for 30 minutes to remove the debris, followed by dehydration in ascendant concentrations of ethanol (50º, 70º, and 95º) for 10 min in each. Then, samples were placed into absolute ethanol for 30 min. After storage in an oven receptacle containing silica for 8 h to remove moisture, the specimens were mounted on an aluminum stub (one stub per group), sputter-coated with a thin layer of gold, and examined with a VEGA 3 LMU scanning electron microscope (TESCAN, Libušina, Czech Republic). The SEM photomicrographs at ×500 magnification was scored individually by five blinded investigators based on the roughness and loss of tooth substance index based on Meyer & Lie, 1977 [25]: (0) - smooth and even root surface without marks from instrumentation and with no loss of tooth substance; (1) - slightly roughened as corrugated local areas confined to the cementum; (2) - definitely corrugated local areas where the cementum may be completely removed, although most of the cementum is still present; (3) - considerable loss of tooth substance with instrumentation marks into the dentin. The cementum is completely removed in large areas or has a considerable number of lesions from the instrumentation. This index was used to qualitatively score the morphology of the root surface produced by each instrument with or without prophylaxis.

Data analysis

Normal distribution of final values of Ra and Rz were analyzed using Shapiro-Wilk and Levene test. The assumption of sphericity of checked using Mauchly's W, and the data of were submitted to Repeated-measures ANOVA. Tukey`s test was used for multiple comparisons. Regarding the scores attributed to SEM images, agreement among the evaluator was assessed by Fleiss Kappa. The score of each specimen was defined as the mode (most observed value) calculated from the scores attributed by the evaluators. Ordinal logistic regression was used to analyze the effect of each factor on scores. A significance level of α = 0.05 was used for all data analyses.

Results

Evaluation of root surface roughness - Ra

Table 1 shows the means and standard deviations observed for each protocol. Data analysis showed that “substrate” ($P < .001$), “instrumentation” ($p < .001$), and “post-instrumentation prophylaxis” ($P < 0.001$) affected the final values of Ra. P-values calculated for all double interactions and for the triple interaction were also significant ($P < 0.001$ for all). In the absence of prophylaxis, the roughest surfaces were observed after ultrasonic instrumentation and in absence of instrumentation, respectively, irrespective the prior irradiation of the substrate. No difference between hand and ultrasonic instrumentation was observed after prophylaxis for both substrates. On the irradiated teeth, the prophylaxis resulted in similar Ra values irrespective of prior instrumentation. The irradiation of substrate affected the Ra values only when the instrumentation (hand or ultrasonic) was not followed by the prophylaxis.

Evaluation of root surface roughness - Rz

Results for Rz are displayed in table 2. RM ANOVA showed that the factors “instrumentation” ($P < .001$), “substrate” ($P < 0.001$), and “post-instrumentation prophylaxis” ($P < 0.001$) affected the final values of Rz. All double interactions and the triple interaction had significant p-values ($P < 0.001$ for all). Irrespective the substrate, in the absence of prophylaxis, the highest values of Rz were observed when the ultrasound was used followed by hand instrumentation. The prophylaxis reduced the Rz values for ultrasonic instrumentation and did not affect the roughness in absence of instrumentation. Smoother surface was obtained with prophylaxis after hand instrumentation only on irradiated substrate. After prophylaxis, no difference on roughness was observed among manual, ultrasonic, and no instrumentation. Irradiating the substrate affected the roughness (rougher surface) only when hand or ultrasonic instrumentations were not followed by prophylaxis.

Scores (SEM images)

Figure 1 and 2 show the surfaces with their respective instrumentation protocols. A moderate concordance among the evaluators was observed (overall Kappa = 0.443). The highest agreement was observed for the score 3 (Kappa = 0.872) and the lowest for the score 1 (Kappa = 0.155). Results of Ordinal logistic regression is presented in Table 3. The substrate did not affect the scores. On the other hand, both manual and ultrasonic instrumentation resulted in higher scores than no instrumentation; and the prophylaxis reduced the scores.

Discussion

In this study, we evaluated changes in the root surface of incisors irradiated or not, caused by manual and ultrasonic instrumentation, associated or not with additional polishing. Our findings demonstrated that the most pronounced surface alterations were observed in the irradiated surfaces, rejecting the null hypothesis.

Different methods have been proposed to analyze changes in the tooth surface after the application of periodontal instruments, including the use of SEM, atomic force microscope, histological evaluation, three-dimensional optical laser scanner, profilometer, and computerized tomography. It is important to emphasize that each technique has its particularities beyond its own limits.[20,21,26,27] In the present study, surface wear was identified using an SEM, while the surface roughness was measured using a contact profilometer device.

Profilometer analysis is a two-dimensional measurement that provides reliable data for characterizing root surface roughness after debridement, and it is one of the most commonly used methods for surface roughness.[19,26,28] The results of the analysis of roughness and root wear associated with the SEM images explain the morphological pattern on the root surfaces found in our study.

Regarding the roughness parameters after instrumentation, Ra and Rz showed that ultrasound instrumentation resulted in the highest values irrespectively of irradiation of tooth substrate. However, for both instruments, the roughest surfaces were observed in the irradiated samples. These results may be related to the possible changes caused by the ionizing radiation on the dental substrate, with alterations in both organic and inorganic contents. Collagen is the most abundant protein in dentin (90%), and its proteolysis significantly impacts the structural integrity of this tissue.[29] Some studies evaluating changes in the chemical composition of the dental structure indicate a drop in the amide I/amide III and amide I/CH_2 ratios. These ratio reductions negatively affect the quality and organization of collagen fibrils and ultimately compromise the physical and mechanical properties of the dentin.[29,30]

Regarding the SEM scores, a moderate agreement between the evaluators was observed. The highest agreement occurred for the score 3, which was indicated for scraping with US where irradiation did not influence. This similarity can be seen by observing images C in figures 1 and 2. It was noted that prophylaxis was able to smooth the rough surfaces (Ra and Rz) observed after hand and ultrasonic instrumentations. This reduction in roughness parameters after prophylaxis corroborates with other studies, and demonstrated its importance for patients more susceptible to periodontal disease and who are underwent radiotherapy of head and neck.[8,14,22,26]

In this study, when performing prophylaxis, it was noticed greater ease of performing in the irradiated groups, so much so that the values of Ra and Rz were lower for these groups. We believe that, besides the changes in organic content, a reduction in concentrations of phosphate and carbonate also reduces the microhardness and elastic modulus of dentin.[29-32] Therefore, a more pronounced effect of instrumentation on surface roughness can be expected in substrates with poorer mechanical properties.[29-32]

An important contribution of this study was to develop an in vitro model that allows controlling as many variables as possible while simulating oral cancer radiotherapy. However, data extrapolation to clinical practice should be done carefully. Several in vitro tests have been used extracted human teeth as substrate. Further to ethical restrictions, difficulties in standardizing this last substrate (e.g., source, age, and other) can impair fairly comparisons. On the other hand, it is easier to standardize the substrate using bovine incisors, which present similar physical and mechanical properties observed in human teeth.[23,31,33] Regarding the storage solutions, physiological saline solution, artificial saliva, or distilled water have been used.[24,32] As the main focus of this study was the direct effect of scaling on irradiated dental tissue, distilled water was chosen. Distilled water provides an environment capable of radiolysis without greater interaction with the teeth.

In conclusion, both manual and ultrasonic instrumentation resulted in rougher root surface irradiated than non-radiated dentin. However, the prophylaxis procedure following the instrumentation significantly reduced the roughness of root surfaces instrumented with either curette or ultrasound. Prophylaxis alone did not significantly change the surface roughness.

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Table 1. Means (standard deviation) of final Ra values (in µm) according to substrate, instrumentation, and post-instrumentation prophylaxis (n = 15).

Substrate		Non-irradiated		Irradiated	
Post-instrumentation prophylaxis		Without	With	Without	With
Instrumentation	Hand	0.31 (0.05) Ba	0.22 (0.04) Aa	0.46 (0.07) Ba*	0.13 (0.04) Ab
	Ultrasonic	1.51 (0.23) Aa	0.25 (0.06) Ab	2.32 (0.25) Aa*	0.20 (0.03) Ab
	None	0.06 (0.01) Ca	0.11 (0.02) Ba	0.05 (0.01) Ca	0.10 (0.02) Aa

For each irradiation, distinct letters (uppercase comparing Instrumentation; lowercase comparing post-instrumentation prophylaxis) indicate statistical difference at Tuckey`s test ($p < .05$). * Indicates statistical difference from non-irradiated substrate for a same instrumentation and post-instrumentation prophylaxis at Tuckey`s test ($p < .05$).

Table 2. Means (standard deviation) of final Rz values (in µm) according to substrate, instrumentation, and post-instrumentation prophylaxis (n = 15).

Substrate	Non-irradiated	Irradiated

Post-instrumentation prophylaxis		Without	With	Without	With
Instrumentation	Hand	1.67 (0.23) Ba	1.10 (0.22) Aa	2.53 (0.48) Ba*	0.69 (0.21) Ab
	Ultrasonic	6.64 (0.87) Aa	0.97 (0.20) Ab	9.63 (1.62) Aa*	1.03 (0.22) Ab
	None	0.34 (0.08) Ca	0.63 (0.09) Aa	0.32 (0.06) Ca	0.57 (0.13) Aa

For each irradiation, distinct letters (uppercase comparing instrumentation; lowercase comparing post-instrumentation prophylaxis) indicate statistical difference at Tuckey`s test ($p < .05$). * Indicates statistical difference from non-irradiated substrate for a same instrumentation and post-instrumentation prophylaxis at Tuckey`s test ($p < .05$).

Table 3. Model coefficients to estimate the scores calculated using the Ordinal logistic regression.

Predictor	Estimate	SE	p-value
Substrate:			
Irradiated vs. Non-irradiated (ref.)	0.031	0.559	0.956
Instrumentation:			
Hand vs. None (ref.)	2.477	0.802	0.002
Ultrasonic vs. None (ref.)	6.221	1.149	< 0.001
Post-instrumentation prophylaxis:			
With vs. Without (ref.)	-3.085	0.713	< 0.001

SE: Standard error.

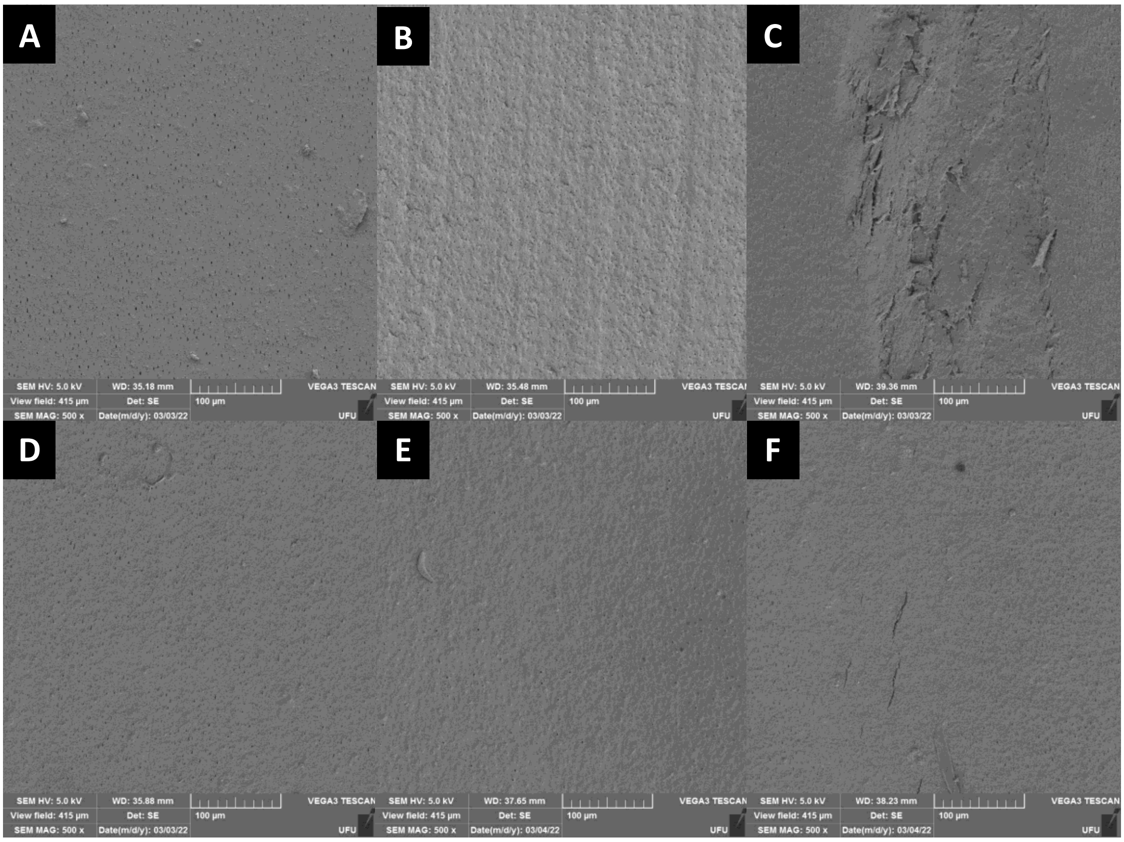

Figure 1. SEM images (500 x) showing the morphology of root surfaces of different interventions of No Irradiated group: (A) No intervention; (B) Scaling with hand instrumentation; (C) Scaling using a ultrasonic instrumentation; (D) Prophylaxis; (E) Scaling with hand instrumentation followed by prophylaxis; (F) Scaling using a ultrasonic instrumentation followed by prophylaxis.

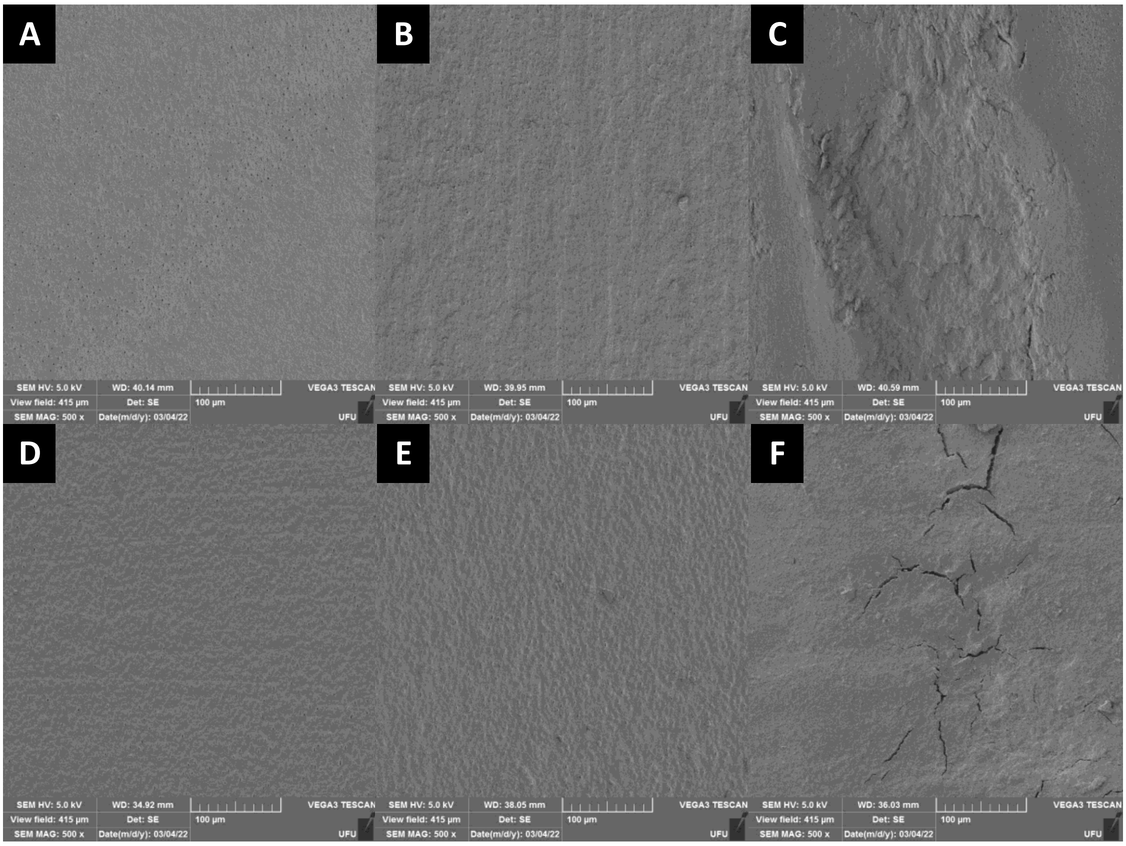

Figure 2. SEM images (500 x) showing the morphology of root surfaces of different interventions of Irradiated group: (A) No intervention; (B) Scaling with hand instrumentation; (C) Scaling using a ultrasonic instrumentation; (D) Prophylaxis; (E) Scaling with hand instrumentation followed by prophylaxis; (F) Scaling using a ultrasonic instrumentation followed by prophylaxis.

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