

# Bleaching and remineralizing agents influence on the microhardness of irradiated dental enamel

Influência de agentes clareadores e remineralizadores na microdureza do esmalte dentário irradiado

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## ABSTRACT

**Objective:** To evaluate *in vitro* the microhardness of bovine dental enamel irradiated with a total dose of 50Gy and bleached with two different gels (one conventional and one containing calcium), and to evaluate whether the use of fluoride remineralizing agents before or after bleaching reduces the effects of radiation and tooth whitening on tooth enamel. **Material and Methods:** 144 bovine teeth were divided into two groups – irradiated (IR, n=72) with a total dose of 50Gy and control (C, n=72). Each group originated nine subgroups (n=8) according to the type of bleaching agent (Whiteness HP Maxx® or Whiteness HP Blue®) and remineralizer used (Desensibilize® KF 2% or Fluor Care® Neutral 2%). Knoop microhardness test and Scanning Electron Microscopy were performed on the specimens. **Results:** Irradiation resulted in statistically lower microhardness means in the bleached groups without remineralizing treatment and in the group that used Desensibilize® before HP Maxx®. The use of Neutral Fluoride increased enamel microhardness by calcium fluoride deposition. Scanning Electron Microscopy showed an increase in roughness and porosity on the enamel surface in the groups that had their microhardness values reduced. **Conclusion:** The use of bleaching gel after irradiation reduced the microhardness of dental enamel, and Neutral Fluoride 2% minimized the effects of the bleaching gel on the irradiated tooth structure.

## KEYWORDS

Dental enamel; Hardness; Radiotherapy; Scanning Electron microscopy; Tooth bleaching.

## RESUMO

**Objetivo:** Avaliar *in vitro* a microdureza do esmalte dental bovino irradiado com uma dose total de 50Gy e clareado com dois diferentes géis (um convencional e um contendo cálcio), e avaliar se o uso de agentes remineralizadores com fluoreto antes ou após o clareamento, diminui os efeitos da radiação e do clareamento dental no esmalte dentário. **Material e Métodos:** 144 dentes bovinos foram divididos em dois grupos – irradiado (IR, n=72) com dose total de 50Gy e controle (C, n=72). Cada grupo originou nove subgrupos (n=8) de acordo com o tipo de agente clareador (Whiteness HP Maxx® ou Whiteness HP Blue®) e remineralizador utilizado (Desensibilize® KF 2% ou Flúor Care® Neutro 2%). Foi realizado teste de microdureza Knoop e Microscopia Eletrônica de Varredura nos corpos de prova. **Resultados:** A irradiação resultou em médias de microdureza estatisticamente inferiores nos grupos clareados sem tratamento remineralizador e no grupo que utilizou Desensibilize® antes do HP Maxx®. O uso do Flúor Neutro aumentou a microdureza do esmalte pela deposição de fluoreto de cálcio. A Microscopia Eletrônica de Varredura mostrou um aumento da rugosidade e porosidade na superfície do esmalte nos grupos que tiveram seus valores de microdureza reduzidos. **Conclusão:** O uso de gel clareador após a irradiação reduziu a microdureza do esmalte dental e o Flúor Neutro 2% minimizou os efeitos do gel clareador na estrutura dental irradiada.

## PALAVRAS-CHAVE

Esmalte dentário; Dureza; Radioterapia; Microscopia eletrônica de varredura; Clareamento dental.

## INTRODUCTION

Head and neck cancer is the seventh most common cancer worldwide [1,2]. Its treatment can be surgical, radiotherapy, chemotherapy or a combination of them, with radiotherapy (RT) being the first choice [3].

RT uses beams of ionizing radiation that produce physicochemical changes in cancerous and healthy cells. Carcinogens, due to their high rate of mitosis, are more susceptible, being led to destruction [1]. Nearby dental tissues may be affected, and there may be a reduction in enamel and dentin microhardness, a reduction in the stability of the bond between the amelodentin junction, loss of the enamel interprismatic structure, a reduction in wear resistance, obliteration of dentinal tubules and atrophy of odontoblastic processes [4-6].

Due to the oral changes that may occur in patients undergoing head and neck radiotherapy, it is important for the dental surgeon to participate in the multidisciplinary team that monitors them. In addition, to improve their self-esteem, the patient can request, after cancer treatment, aesthetic treatments such as tooth whitening, since the smile is an important factor in socializing between people and its improvement can increase their self-image, self-confidence and quality of life [7,8].

Despite being a known and widely used procedure, bleaching agents can lead to a decrease in surface microhardness, an increase in porosity and roughness, changes in morphology, erosion and an increase in permeability due to demineralization of the dental substrate. These changes are directly associated with the concentration of the gel, its pH, its composition, the time of use and the etiology of the stains [9,10].

Calcium and fluorine have been added to the composition of bleaching agents and lead to a smaller reduction in enamel microhardness compared to conventional bleaching agents [11,12]. For this purpose, it is also proposed to use remineralizing agents, such as fluorides [7,9,13-15]. Since they promote calcium phosphate precipitation in dental tissues, reducing the effects of hydrogen peroxide on enamel [10].

The analysis of the influence of the effects of bleaching agents and irradiation on the dental structure, especially their sum, is necessary, since both can lead to a decrease in enamel surface microhardness, an increase in roughness and morphology changes. Thus, the objectives of this study were: to evaluate *in vitro* the microhardness of bovine dental enamel irradiated with a total dose of 50Gy and bleached with two different gels, one conventional and one containing calcium in its composition; and to assess whether the use of remineralizing agents with 2% fluoride concentrations, before or after bleaching, reduces the effects of radiation and tooth bleaching on tooth enamel. The experimental hypotheses are: (1) the ionizing radiation used in head and neck radiotherapy reduces the microhardness of dental enamel; (2) performing tooth whitening after radiotherapy can increase this microhardness reduction effect; (3) the use of remineralizing agents, before or after the bleaching procedure, can minimize these effects on enamel microhardness.

## MATERIALS AND METHODS

A total of 144 healthy bovine permanent incisors of cattle were selected. Cleaning and disinfection in 0.1% thymol solution were performed for 48 hours, followed by hydration in saline solution for five days. Storage was in distilled water and oven (37°C).

They were randomly divided into two groups, irradiated (group IR, n=72) and control (group C, n=72). The IR group was submitted to ionizing radiation in a radiotherapy clinic located in the city of Lauro de Freitas, Bahia. The protocol for the treatment of head and neck tumors was followed, which consists of exposure to X-rays of 6 MV, in a linear accelerator (Elekta Ltd., West Sussex, United Kingdom), in a fractional regimen of 2Gy, for 5 consecutive days, with a break of 2 days, until the total dose of 50Gy, being performed 25 cycles of 2Gy during 5 weeks. This total dose was stipulated considering studies that indicate that this is one of the most frequent exposure ranges for treating tumors found in the oral cavity, falling within the therapeutic range of 50 to 70 Gy [16]. This analysis was performed

using computed tomography simulating the treatment of a tongue tumor. The teeth were exposed with their long axis parallel to the ground in an acrylic box (20 x 20 x 10 cm) with distilled water to maintain moisture. The incidence of the radiation beam was perpendicular to the box using the isocentric technique.

The teeth were stored in distilled water and oven at 37°C and, after four weeks of radiotherapy, the whitening protocols were started. The groups were divided into nine subgroups (n=8), according to the bleaching agent type (Whiteness HP Maxx® and Whiteness HP Blue® - FGM Dental Group Ltd., Joinville, SC, Brazil) and used remineralizer (Desensibilize® KF 2% and Fluor Care® in Neutral foam 2% - FGM Dental Group Ltda., Joinville, SC, Brazil). This use followed the scheme shown in Table I.

The materials were applied to the buccal surface of the tooth crowns according to the manufacturer's recommendations (Tables II), following the in-office bleaching protocol, performed for 3 consecutive weeks, with 7-day intervals between applications, as well as remineralizing agents. Desensibilize® KF 2% was applied for 10 minutes before the bleaching gel, and Fluor Care® Neutral 2% was applied for 1 minute after the bleaching gel was removed. Storage was in distilled water and oven at 37°C.

After application of the materials, the crown of the teeth was sectioned in its incisal third, on the buccal surface, with a double-sided diamond disc (American Burrs® - Palhoça, SC, Brazil) coupled in a straight piece, under refrigeration, to obtain fragments measuring 6mm x 6mm x 2mm and stored in distilled water at 37°C.

For the Knoop microhardness reading, three indentations were performed on each specimen with a microhardness tester (HNV-G - Shimadzu Corporation, Kyoto, Japan), with a load of 0.05HK (490.3mN) applied for 15 seconds. The hardness value of each specimen was calculated from the arithmetic mean of the values obtained in the three indentations.

For surface evaluation by scanning electron microscopy (SEM), two random samples from each group were selected and previously adapted to a carbon tape adhered to a metallic stub, where they were stored in an airtight pot with silica gel in an oven at 37°C for 24h for the excess water to be removed and the samples to be completely

dry for evaluation. After 24h, the samples were removed from the oven and coated with gold. Then, the surface evaluation equipment (JSM 5600LV – Jeol Inc., Peabody, MA, USA) was operated at 15 kV, with surface images captured at 1000X magnification. Exploratory analysis of microhardness data was performed to verify normality in the distribution between groups (Shapiro-Wilk;  $p > 0.05$ ) and other parameters of analysis of variance (ANOVA). The inferential statistical analysis of the data obtained was performed by ANOVA in a factorial scheme (9x2), "INTERVENTION" x "IRRADIATION". Tukey's post-hoc test was used for multiple comparisons between means. The analyzes were performed using the SAS 9.1 statistical program, with a significance level of 5%.

## RESULTS

According to the statistical analysis of the Knoop microhardness data, a significant interaction was observed between the two factors studied, which indicates dependence between them ( $p = 0.0066$ ). This interaction was unfolded by Tukey's test and is described in Table III.

When the interventions are compared, in the absence of irradiation, it is noted that the mean hardness of the group without intervention (C1) was statistically higher than that of the DESENSIBILIZE 2% + HP MAXX group (C6). The

**Table I** - Division of study groups IR and C according to the bleaching gel and remineralizing agent used

Grupo IR (n=72)	Grupo C (n=72)
IR1- Control (n=8)	C1- Control (n=8)
IR2- Desensibilize KF 2% (n=8)	C2- Desensibilize KF 2% (n=8)
IR3- Neutral Fluoride 2% (n=8)	C3- Neutral Fluoride 2% (n=8)
IR4- Whiteness HP Maxx (n=8)	C4- Whiteness HP Maxx (n=8)
IR5- Whiteness HP Blue (n=8)	C5- Whiteness HP Blue (n=8)
IR6- Desensibilize KF 2% + Whiteness HP Maxx (n=8)	C6- Desensibilize KF 2% + Whiteness HP Maxx (n=8)
IR7- Whiteness HP Maxx + Neutral Fluoride 2% (n=8)	C7- Whiteness HP Maxx + Neutral Fluoride 2% (n=8)
IR8- Desensibilize KF 2% + Whiteness HP Blue (n=8)	C8- Desensibilize KF 2% + Whiteness HP Blue (n=8)
IR9- Whiteness HP Blue + Neutral Fluoride 2% (n=8)	C9- Whiteness HP Blue + Neutral Fluoride 2% (n=8)

**Table II** - Composition and instructions for use of the whitening gels and the remineralizing agents manufacturer

Whitening gel	Composition	Instructions for use
Whiteness HP Maxx® (FGM Dental products Ltda., Joinville, SC, Brasil)	35% hydrogen peroxide, thickeners, dye mixture, glycol, inorganic filler and deionized water.	Shake the thickener bottle. Add 3 drops of hydrogen peroxide to 1 drop of thickener and mix. Cover the entire buccal surface of the teeth to be whitened with a layer of gel 0.5 to 1 mm thick. Let the gel act for 15 minutes. With the aid of a microapplicator, move the gel three to four times. Suck the gel with a suction cannula, clean the teeth with gauze and apply the gel again. Perform three 15-minute applications. After the last application, wash your teeth with water. Repeat the process after an interval of 7 days.
Whiteness HP Blue® (FGM Dental products Ltda., Joinville, SC, Brasil)	35% hydrogen peroxide, thickeners, inert violet pigment, neutralizing agents, calcium gluconate, glycol and deionized water.	Mix the two phases with the syringes connected, pushing the plungers alternately for up to 8 times; push the entire contents mixed into one of the syringes. Attach a tip to the syringe with the gel and apply a layer 0.5 to 1 mm thick over the entire buccal surface of the teeth to be whitened. Let the gel act for 40 minutes. With the help of the microbrush, move the gel over the teeth every 5 or 10 minutes. After 40 minutes, aspirate the gel with a cannula and wash the teeth with plenty of water. Repeat the procedure after 7 days.
Desensibilize® KF 2% (FGM Dental products Ltda., Joinville, SC, Brasil)	5% potassium nitrate and 2% sodium fluoride, deionized water, glycerin, neutralizing and thickening agent	Clean the surface of the tooth, apply the gel evenly over the teeth, wait for 10 minutes to act. Remove the gel with gauze and plenty of water.
Flúor Care® Neutral 2% in tutti-frutti foam (FGM Dental products Ltda., Joinville, SC, Brasil)	2% sodium fluoride	Clean the surface and apply the foam to the tooth. Wait 1 minute and remove excess with gauze.

other groups showed intermediate means, with no statistically significant difference between them. In the presence of irradiation, the mean of the group without intervention (RI1) was statistically higher than the mean of the HP MAXX (IR4), HP BLUE (IR5) and DESENSIBILIZE 2% + HP MAXX (IR6) groups. The others showed intermediate values, without significant differences.

Irradiation resulted in statistically lower Knoop microhardness averages for groups bleached with HP MAXX and HP BLUE products, which did not receive any remineralizing treatment. In the other groups, there was no difference between the means obtained with and without irradiation.

Through the images obtained by Scanning Electron Microscopy of the study groups, it is possible to observe that the use of Desensibilize® KF 2% prior to Whiteness HP Maxx® caused an increase in roughness and porosity on the enamel surface Figure 1.

When comparing the irradiated groups, those bleached (IR4 and IR5) and the one that used Desensibilize® KF 2% previously to Whiteness HP Maxx® (IR6) showed greater roughness and porosity when compared to the IR1 group, and these changes were more marked in the IR6 group (Figure 2).

The irradiated groups that were bleached (IR4 and IR5) showed higher roughness and porosity when compared to the control groups (C4 and C5) as shown in Figure 3.

In Figure 4 it is possible to compare the surface of all the control groups studied, and in Figure 5, the irradiated groups can be compared.

**DISCUSSION**

In the present study, bovine incisors were used due to their similarity with human enamel in terms of hardness and chemical and biological composition [17]. The teeth were stored in distilled water, because during irradiation, these medium favors radiolysis and does not have additional interactions with the dental substrate [18]. In addition, this storage allows a more specific assessment of the influence of bleaching and remineralizing agents without the interference of saliva, since, due to its remineralizing potential, it could be a confounding fator [13].

The first hypothesis of the present study – that the ionizing radiation used in head and neck radiotherapy reduces the microhardness of dental enamel – was denied, since the group that received the irradiation did not show a significant difference in the Knoop microhardness of non-irradiated



**Table III** - Mean (standard deviation) of Knoop microhardness measured in the experimental groups (kgf/mm<sup>2</sup>)

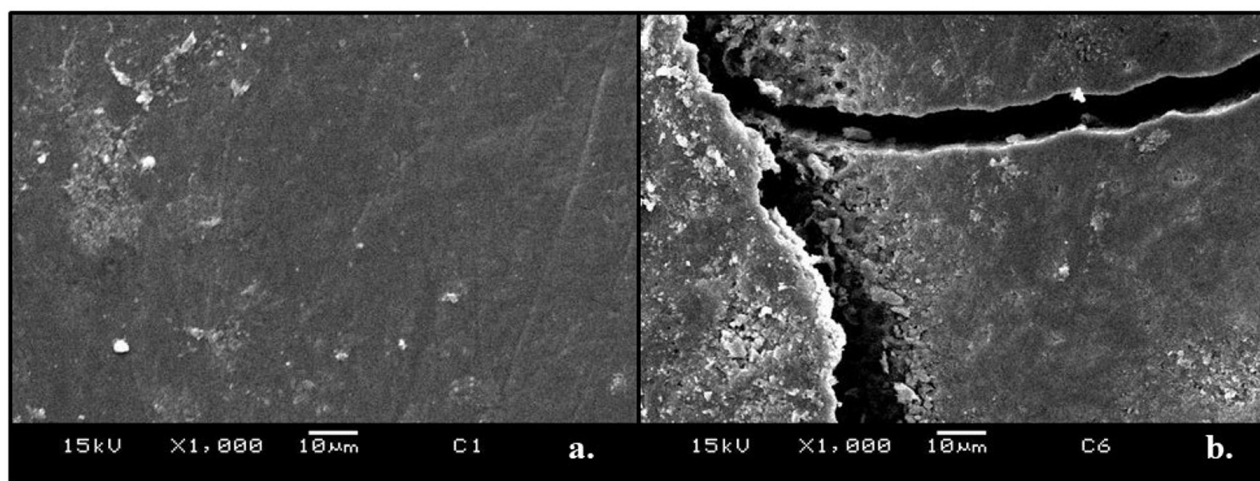
INTERVENTION	IRRADIATION			
	ABSENT		PRESENT	
1. W/O INTERVENTION	352.08	(79.81) Aa	358.66	(110.67) Aa
2. DESENSIBILIZE KF 2%	277.04	(97.41) ABa	288.16	(78.67) ABa
3. NEUTRAL FLUORINE 2%	275.95	(93.74) ABa	262.41	(61.58) ABa
4. WHITENESS HP MAXX	334.45	(56.03) ABa	198.33	(74.09) Bb
5. WHITENESS HP BLUE	314.50	(27.50) ABa	170.97	(72.75) Bb
6. DESENSIBILIZE 2% + HP MAXX	205.41	(69.95) Ba	182.83	(49.33) Ba
7. HP MAXX + FLUORINE 2%	325.20	(88.68) ABa	312.75	(66.59) ABa
8. DESENSIBILIZE 2% + HP BLUE	262.75	(72.22) ABa	250.41	(58.97) ABa
9. HP BLUE + FLUORINE 2%	243.87	(56.29) ABa	275.53	(94.35) ABa

**Note:** Means followed by different letters represent statistical significance (2-way ANOVA /Tukey;  $\alpha=5\%$ ). Capital letters compare differences between INTERVENTIONS within each irradiation level. Lowercase letters compare the IRRADIATION factor within each intervention level.

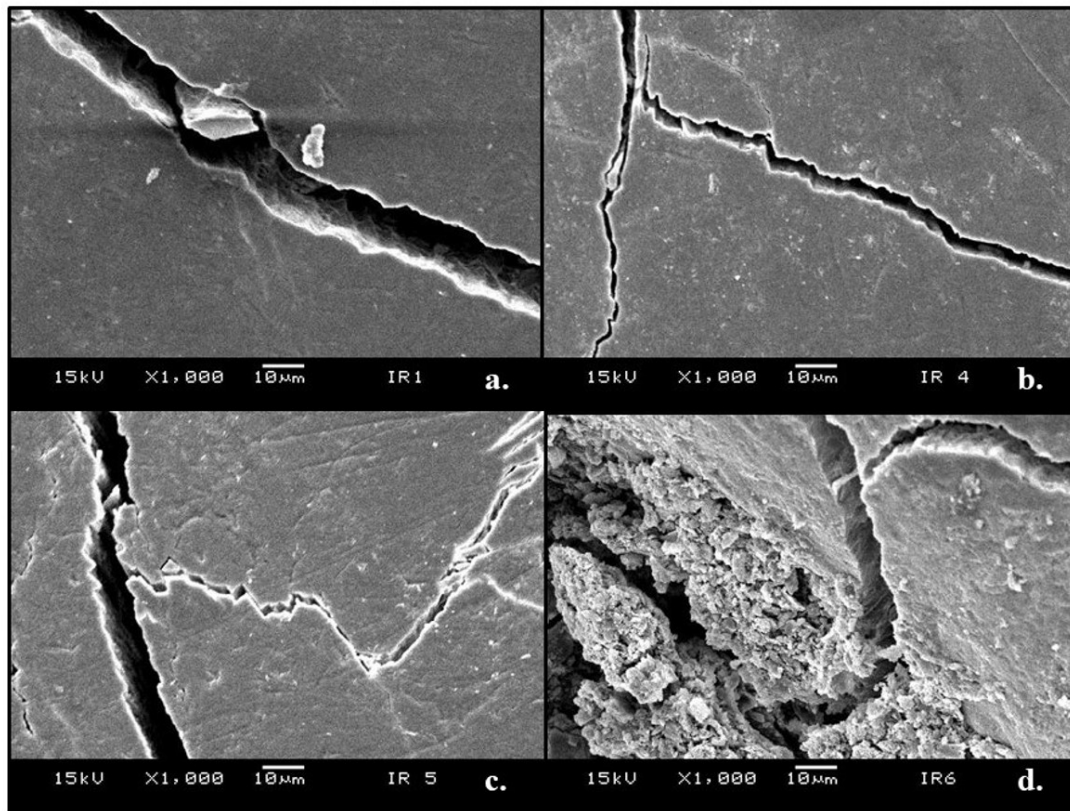
bovine enamel. Cunha et al. [18], when evaluating the influence of different doses of radiation on the enamel microhardness of the occlusal, middle and cervical thirds, observed that only the enamel of the cervical third presented reduced microhardness values, due to its greater porosity and smaller thickness, with minimal changes in the microhardness of the enamel on the other thirds. The minimum dose that led to changes in tooth structure was 50Gy. The present study used a dose of 50Gy and evaluated the enamel microhardness of the incisal third, with no significant changes being found on the enamel surface. According to Liang et al. [19], damage to the dental structure is directly related to the dose provided, and changes, such as reduction in nanohardness and elastic modulus, were significant when exposed to doses of 50Gy. Doses above this had little additional effect on the changes found.

*In vitro* studies have demonstrated a reduction in enamel microhardness after it is subjected to ionizing radiation [18-20]. This may occur due to the decarboxylation of collagen carboxylate lateral bonds that are responsible for the interaction of the enamel organic matrix with the apatite crystals and, with their breakage, there is a reduction in the mineral-organic interaction, which provides greater fragility to the tissue. This alteration can affect the interprismatic region of the enamel, since it is where a large amount of proteins and water are concentrated [20].

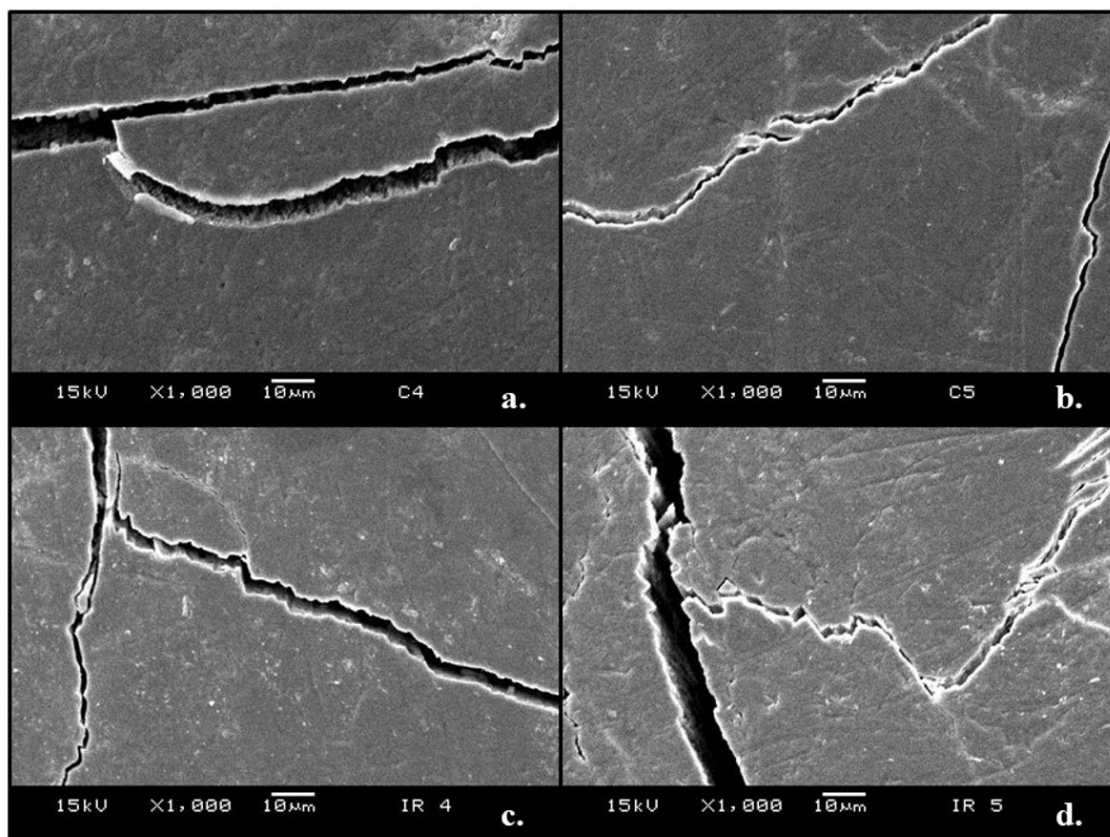
However, in the study of Gonçalves et al. [4], ionizing radiation promoted an increase in enamel microhardness after a dose of 40Gy and a reduction in dentin microhardness only. The difference in the behavior of these two substrates against ionizing radiation may be linked to the fact that radiation acts by radiolysis, with



**Figure 1** - Scanning Electron Microscopy (1000x). a. Group C1. b. Group C6. The use of Desensibilize KF 2% + Whiteness HP Maxx led to an increase in roughness and porosity on the enamel surface.

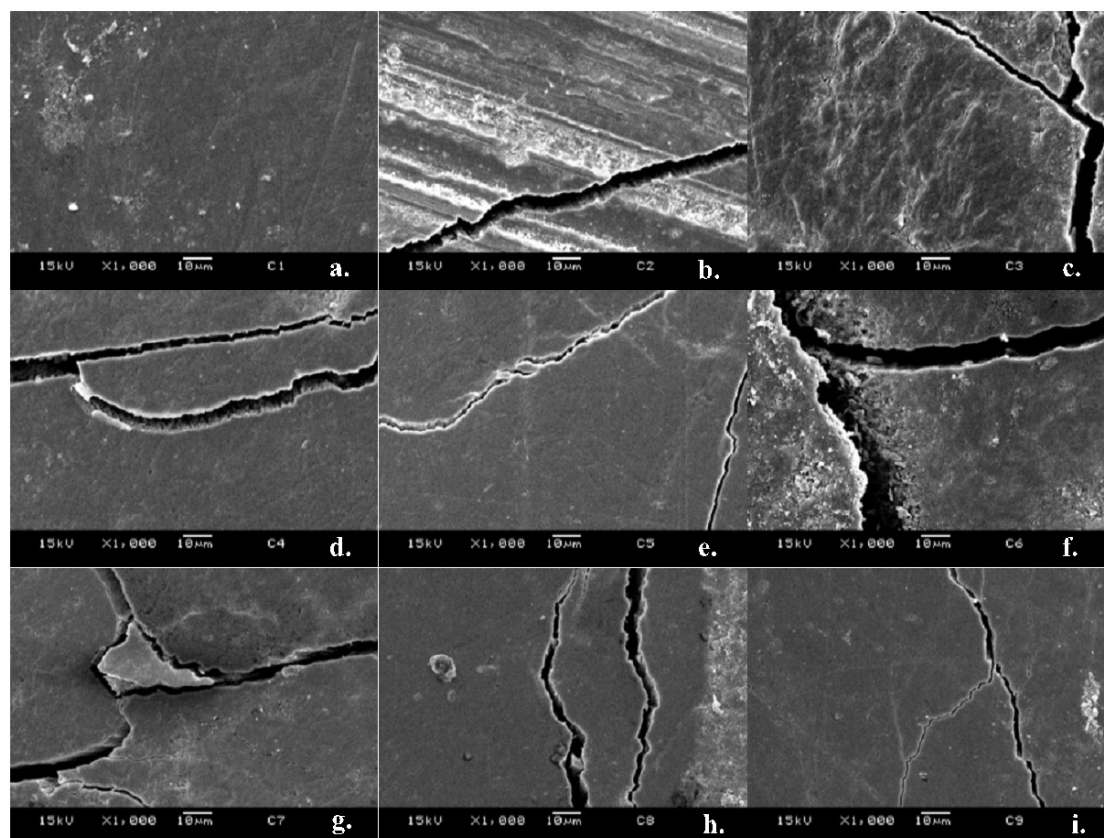


**Figure 2** - Scanning Electron Microscopy (1000x). a. Group IR1. b. Group IR4. c. Group IR5. d. Group IR6. The use of bleaching gels after irradiation led to greater roughness and porosity on the enamel surface, being more pronounced in the group that used Desensibilize KF 2% + Whiteness HP Maxx (IR6).

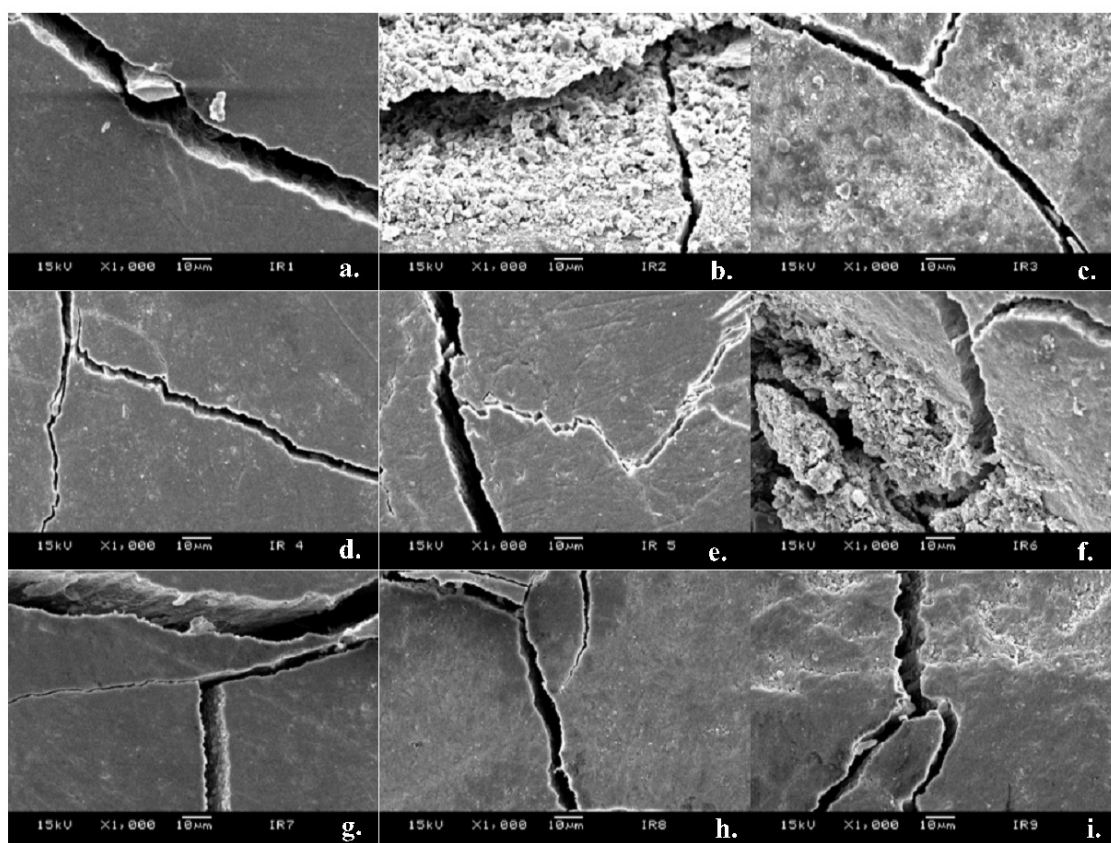


**Figure 3** - Scanning Electron Microscopy (1000x). a. Group C4. b. Group C5. c. Group IR4. d. Group IR5. Irradiation prior to the use of bleaching gels generated more roughness and porosity on the enamel surface.





**Figure 4** - Scanning Electron Microscopy (1000x). a. Group C1. b. Group C2. c. Group C3. d. Group C4. e. Group C5. f. Group C6. g. Group C7. h. Group C8. i. Group C9. Comparison of the surface of the control groups.



**Figure 5** - Scanning Electron Microscopy (1000x). a. Group IR1. b. Group IR2. c. Group IR3. d. Group IR4. e. Group IR5. f. Group IR6. g. Group IR7. h. Group IR8. i. Group IR9. Comparison of the surface of the irradiated groups.

greater formation of free radicals and hydrogen peroxide where there is greater presence of water. As dentin has a higher water content than enamel, the greatest changes occur in it. The SEM analysis showed a greater degradation of the interprismatic portion of the enamel after doses of 30 and 60 Gy, probably because it is where there is a greater concentration of water.

Studies measuring the influence of radiation and whitening gel on dentin are important, since this substrate is more susceptible to radiation and the whitening gel acts on it through diffusion

The second hypothesis – that performing tooth whitening after radiotherapy can increase the microhardness-reducing effect – was fully confirmed. Irradiated and subsequently bleached bovine enamel showed significantly lower microhardness than the control group. A possible explanation is that the irradiation, when affecting the interprismatic portion of the enamel [4], may have left this region more susceptible to the effects of the whitening gel, which acts by diffusion on the tooth structure, leading to changes such as depressions and erosions, as demonstrated in the study by Coceska et al. [10]. Possibly, there was a cumulative effect of changes in the enamel, initially by irradiation, which alone did not have a great effect, but, followed by contact with the bleaching gel, led to a significant reduction in microhardness.

The third hypothesis – that the use of remineralizing agents, before or after the bleaching procedure, can minimize the effects on enamel microhardness – was partially accepted, since only the post-bleaching application of 2% neutral fluoride was effective in increasing microhardness of the enamel surface irradiated and bleached enamel with both bleaching gels. Gomes et al. [7] also did not obtain an improvement in the microhardness of the enamel with the use of remineralizing agents before tooth whitening.

However, it should be noted that, despite the two products having the same fluoride concentration (2%), they were applied at different times and for different times. Fluor Care® Neutral 2% was applied for one minute after removing the whitening gel, due to its action as a remineralizer. It acts by deposition of calcium fluoride on the enamel surface with the consequent obliteration of the dentinal tubules. Desensibilize® KF 2% was applied before the whitening gel for ten minutes. It has 2% fluoride and 5% potassium nitrate, which decrease the ability of nerve fibers in the dental

pulp to repolarize after depolarization, blocking nerve activity and reducing sensitivity. Their use did not significantly affect the microhardness of bovine enamel when used alone. This result differs from previous studies that show the effectiveness of remineralizers in improving enamel microhardness [13-15,20-22].

In the control groups, there was a statistically significant reduction in enamel microhardness in the group that used Desensibilize® followed by HP Maxx® (C6), when compared to that observed in the group without intervention (C1). This demonstrates that the whitening agent reduced enamel microhardness, and that the remineralizing agent, when applied before whitening, was not able to prevent this effect. According to information from the manufacturer, the pH of this agent ranges from 5 to 7, and values below 5.5 can already lead to demineralization of the enamel surface. This result differs from the findings of Alencar et al. [23] who demonstrated that the use of 5% potassium nitrate with 2% fluoride was able to increase enamel microhardness, which was reduced by the application of 35% hydrogen peroxide, which was applied after whitening. These findings possibly differed from the current study due to the timing of the product application.

The group in which Whiteness HP Maxx® and Fluor Care® 2% (C7) were used showed microhardness averages closer to the hardness averages of the C1 group. The fact that its application was performed after the use of the whitening gel may have maintained the calcium fluoride layer on the enamel surface, contributing to the maintenance of dental enamel hardness levels.

Neutral fluoride at 2% was chosen because it promoted an increase in enamel microhardness, and acidulated fluorophosphate (FFA) reduced when both were associated with 35% hydrogen peroxide and compared to the use of peroxide alone. The authors attribute this to the fact that the low pH of the FFA favors the demineralization of the structure [15]. The groups that used neutral fluoride maintained the microhardness parameters of the irradiated and unbleached groups as in the control groups, proving to be an interesting option for association with bleaching treatment, especially for irradiated teeth.

In the control groups in which Whiteness HP Blue® was used alone or associated with Desensibilize® or 2% neutral fluoride, there was no significant reduction in microhardness. The



presence of calcium in its composition and the pH stability of the gel may have contributed to this finding. The incorporation of calcium into the bleaching agent has been an important factor in reducing changes in the bleaching gel in tooth enamel [12-14,24]. The use of Whiteness HP Blue® also prevented changes in tooth enamel microhardness without reducing the effectiveness of bleaching in Alexandrino's et al. [25] research.

Mendonça et al. [26], evaluated the pH of these whitening products and observed that Whiteness HP Maxx starts whitening with an average pH of 6.05 and at the end of its application, this pH becomes around 5.16. Therefore, this whitening product has characteristics of an acidic gel, which can lead to greater demineralization of the tooth structure. Whiteness HP Blue, on the other hand, starts with a pH of 8.54 and after 40 minutes of application, this pH is 8.37 on average, showing characteristics of alkalinity and pH stability throughout the whitening process. Gels with a more alkaline pH and that can maintain the stability of this pH during their application, lead to less demineralization of the tooth structure, since it starts with a pH below 5.5.

When comparing the bleached groups (IR4, IR5, C4 and C5), it is observed that irradiation significantly influenced the reduction of enamel microhardness. Therefore, dental surgeons should be cautious when performing bleaching treatment in patients who have been submitted to ionizing radiation, as they are more susceptible to reducing the microhardness of dental enamel. And, after using the whitening gel, they must apply remineralizing agents, such as 2% neutral fluoride to recover the damage.

However, more *in vitro* studies, including with saliva, and clinical studies should be carried out to confirm the findings of this study and to enable a more reliable clinical evaluation of the variables studied, thus helping dental surgeons to perform the bleaching treatment in patients undergoing radiotherapy safely. Furthermore, using bovine teeth instead of human teeth may lead to differences in results.

According to the results obtained, it can be concluded that radiotherapy alone did not influence the microhardness of dental enamel; the use of bleaching gel after radiotherapy significantly reduced the microhardness of dental enamel; Neutral Fluoride 2% proved to be effective in increasing enamel microhardness after tooth bleaching, being

an option to minimize the effects of bleaching gel; 2% Desensibilize KF was not effective in increasing the microhardness of irradiated enamel.

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## Author's Contributions

TOGS: Conceptualization, Methodology and Writing – Original Draft Preparation. MAGA: Methodology. JEDS: Methodology. LV: Writing – Review & Editing. ANC: Data Curation. PM: Conceptualization, Visualization and Supervision.

## Conflict of Interest

No conflicts of interest declared concerning the publication of this article. The materials tested were kindly provided by FGM Dental Group, without any influence on the results or interpretations presented.

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## Regulatory Statement

Research involving bovine teeth does not require submission to the Ethics Committee on the Use of Animals (CEUA).

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