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Original Research

Effect of brushing with different toothpastes on irradiated enamel roughness and composition: an in vitro study

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ABSTRACT

Objectives: To evaluate the effect of toothbrushing with different toothpastes on the roughness and mineral composition of irradiated and non-irradiated enamel.
Methods: One hundred twenty bovine crowns were randomly divided into three irradiated and non-irradiated groups submitted to toothbrushing with Bianco Pro Clinical 1% TCP, Colgate Sensitive Pro-Relief, or Sensodyne Sensitivity & Gum (n = 20 per group). Irradiation was performed in fractionated doses, totaling 72 Gy. All specimens were brushed in a 30-day simulation cycle. Surface roughness (Ra-µm) and chemical composition were measured before and after toothbrushing. Data were analyzed using the two-way ANOVA followed by Tukey's test with a significance level of α=0.05.
Results: Irradiated enamel exhibited greater roughness than non-irradiated, even before toothbrushing, and had higher levels of amide I and phosphate but lower carbonate content. After toothbrushing, roughness increased in the irradiated enamel, regardless of the toothpaste used. Post-brushing changes in enamel composition varied depending on the toothpaste. Sensodyne and Colgate caused greater reductions in amide I compared to Bianco. Irradiated enamel treated with Bianco showed increased carbonate content, while those brushed with Colgate exhibited higher phosphate V1-V3 levels.
Conclusions: Irradiation increased enamel roughness, and toothbrushing further amplified this effect, with no significant differences observed among the evaluated toothpastes. Both irradiation and toothbrushing influenced the enamel's chemical composition, with changes varying based on the specific toothpaste used. (Rev Port Estomatol Med Dent Cir Maxilofac. 2025;66(3):108-114)

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Introduction

Cancer remains one of the most significant global health challenges, with head and neck malignancies affecting millions of people each year.[1-3] Radiotherapy, which is primarily used to shrink tumors and eliminate cancer cells,[4] has contributed to improving survival rates. However, it also has adverse short- and long-term effects on oral health,[3] leading to complications that can significantly compromise oral care in cancer patients.[5]

One of the primary concerns with radiotherapy is the damage it causes to healthy tissues surrounding the tumor, including the salivary glands and oral mucosa, resulting in hyposalivation.[4] This dry oral environment impacts patient comfort and increases the risk of caries lesions and periodontal disease.[1,6] Despite these risks, preventive strategies, such as evaluating and providing baseline oral care before radiotherapy, are often overlooked.[2,7]

Radiation exposure can also lead to detectable changes in enamel structure, including alterations in its mechanical and chemical properties.[8,9] Specifically, irradiated teeth have been found to exhibit reduced enamel hardness, particularly near the cement-enamel junction, compared to non-irradiated teeth.[10,11] This reduction in hardness makes enamel more susceptible to wear and fractures, contributing to the development of radiation-related caries.[9,10] Additionally, radiation may cause changes in the enamel's chemical composition, particularly in its carbonate content, further compromising structural integrity.[9] These findings emphasize the importance of comprehensive dental care during and after radiotherapy to preserve oral health.[12,13]

Proper toothbrushing techniques are effective not only in removing plaque and reducing bacterial count but also in preserving dental enamel integrity.[14,15] However, toothpaste composition can influence enamel abrasion, especially in enamel altered by irradiation. The impact of different toothpastes on irradiated enamel remains unclear. Thus, this study aimed to evaluate the effect of different toothpastes on surface roughness and chemical composition changes in irradiated and non-irradiated bovine enamel. The null hypothesis tested was that neither the type of toothpaste nor irradiation would influence the changes in roughness or chemical composition resulting from toothbrushing.

Material and methods

This in vitro study assessed two independent variables: irradiation at two levels—non-irradiated (NIR) and irradiated (IR); and toothpaste at three levels—Bianco Pro Clinical 1% TCP (Bianco, Uberlândia, Brazil), Colgate Sensitive Pro-Relief (Colgate, New York, USA), and Sensodyne Sensitivity & Gum (Sensodyne, Seoul, South Korea) (Table 1). The dependent variables evaluated included roughness (measured in Ra) and enamel composition in terms of amide I, phosphate V3-1, carbonate, and phosphate contents. These variables' measured values were assessed both before and after toothbrushing. Only the levels of irradiation were compared for the data collected before brushing. In contrast, the effects of both irradiation and toothpaste were evaluated for the changes caused by toothbrushing.

One hundred twenty bovine teeth were selected for this study. The crowns were separated from the roots by sectioning at the cementoenamel junction using a low-speed micromotor and a diamond disc (American Burrs, Palhoça, SC, Brazil). The crowns were stored at 4°C in distilled water, which was replaced weekly until the specimens were prepared.

Each crown was embedded in a polystyrene resin cylinder (Cristal, Piracicaba, SP, Brazil) with a diameter of 20 mm. The buccal enamel surface was then flattened and polished using 600-, 800-, 1200-, and 2000-grit silicon carbide papers (Norton, Campinas, SP, Brazil), followed by polishing with a felt disc and metallographic diamond pastes (6, 3, 1, and 1/4 µm; Arotec, São Paulo, SP, Brazil). The samples were cleaned with three 10-minute ultrasonic baths (Cristofoli, Campo Mourão, Paraná, Brazil) in distilled water to remove any debris.

Half of the specimens were randomly selected for irradiation and were immersed in distilled water at room temperature during the irradiation protocol, as this solution facilitates radiolysis without significantly interacting with dental tissues. Fractional radiation was administered at a dose of 1.8 Gy per fraction per day, five days a week, for eight weeks, resulting in a total dose of 72 Gy. This was achieved using X-rays from a linear accelerator (Clinac 600C Varian®, Palo Alto, CA, USA, Beam 6 MV). The specimens in the NIR group were similarly immersed in deionized water, which was changed daily.[16]

The enamel's surface roughness (Ra, µm) for both NIR and IR specimens was measured using a profilometer (Surftest SJ-301, Mitutoyo, Japan). Five parallel readings were taken in a predetermined area, and the average value was calculated. Measurements were conducted with a cut-off of 0.25 mm and a measurement length of 1.25 mm at a speed of 1 mm/s, covering a total distance of 3 mm.

The chemical composition of all samples was determined using a Fourier-transform infrared spectrometer (FTIR) (Vertex 70, Bruker, Ettlingen, Germany) employing the attenuated total reflectance (ATR) technique. The enamel surface was placed in contact with the diamond crystal of the ATR unit and was continuously pressed by the equipment's claw. Spectra were

Table 1. Toothpastes tested in this study.

Toothpaste	Main component	Manufacturer
Bianco Pro Clinical 1% TCP	Tricalcium phosphate 3%	Bianco Oral Care, Uberlândia, MG, Brazil
Colgate Sensitive Pro-Alivio	Arginine 8%, sodium monofluorophosphate 1.10%	Colgate-Palmolive Company, New York, NY, United States
Sensodyne Sensibilidade & Gengivas	Stannous fluoride 0.072%, sodium fluoride (1450 ppm of fluoride)	GlaxoSmithKline Korea Ltd, Seoul, South Korea

obtained in the range of 400 to 4000 cm^{-1}, with a resolution of 4 cm^{-1}. Each sample was scanned 32 times, and the final spectrum was the average of all scans.[8,16,17] The spectra were recorded and analyzed using OPUS 6.5 software (Bruker, Ettlingen, Germany). After baseline adjustment, the area under each spectral band was calculated using the software's specific tools. The analyzed bands included amide I (1575–1730 cm^{-1}), carbonate v^3 (1350–1520 cm^{-1}), phosphate v1 and v3 (958 and 1040 cm^{-1}), and phosphate (1190–702 cm^{-1}).[8,17]

The NIR and IR specimens were randomly divided for simulated brushing with one of the three tested types of toothpaste (n = 20). The specimens were fixed in a simulated dental brushing machine (Odeme Dental Research, Luzerna, SC, Brazil). A mixture of toothpaste and artificial saliva (2:1 ratio, 8 g of toothpaste to 4 mL of saliva per specimen) was dispensed onto the matrix to cover the specimen surface. Soft-bristled toothbrushes (Bianco Colorcare, Lima & Pergher Comércio S/A, Uberlândia, Brazil) had their heads removed and attached to the device. The specimens underwent mechanical brushing in a simulated daily brushing scenario for 30 days,[18] with linear motion, 2 minutes of rest time, a vertical load of 200 g on the toothbrush heads, and a temperature of 25 ± 1°C.[19] After each brushing cycle, the specimens were washed with three 10-minute ultrasonic baths (Cristofoli, Campo Mourão, Paraná, Brazil) in distilled water to remove debris. The toothpaste mixture was replaced after each brushing cycle, and the brushing machine was cleaned. After the toothbrushing procedures, the roughness and chemical composition of the enamel were measured again using the same protocol as before.

Data normality was assessed using the Shapiro-Wilk test, while equal variance was evaluated with Levene's test. At baseline, the data for each outcome from the NIR and IR specimens were compared using a *t*-test. The outcome changes resulting from the toothbrushing procedures were calculated by subtracting the initial values from the final measurements. Subsequently, changes in roughness and enamel composition were analyzed using a two-way ANOVA (irradiation vs. toothpaste), followed by Tukey's test for post-hoc comparisons. A confidence level of 95% was established for all data analyses.

Results

The baseline measurements of surface roughness and enamel components are presented in Table 2. The enamel exhibited greater roughness in the IR specimens than in the control (NIR). Except for phosphate v1-v3, irradiation significantly affected the enamel components by increasing amide I and phosphate levels while reducing carbonate content.

Mean values of Ra changes and standard deviations for IR and NIR enamel treated with different toothpastes are shown in Table 3. The two-way ANOVA revealed a significant effect for the irradiation factor (p = 0.001), while the toothpaste factor (p = 0.249) and the interaction between both factors (p = 0.527) were not significant. Regardless of the toothpaste used, toothbrushing led to a greater roughness increase in the IR groups' enamel compared to the NIR groups.

Mean values and standard deviations for the changes in enamel components in IR and NIR enamel treated with different toothpastes are presented in Table 4. Amide I levels were significantly influenced by both irradiation ($p < 0.001$) and toothpaste (p = 0.033), while the interaction between these factors was not significant (p = 0.505). The IR groups exhibited a greater reduction in amide I content than the NIR groups, regardless of the toothpaste used. Specifically, Sensodyne and Colgate produced similar and greater reductions in amide I compared to Bianco toothpaste. Notably, the amide I content significantly increased only when NIR specimens were brushed with Bianco.

Regarding carbonate content variation, both independent variables—irradiation (p = 0.043) and toothpaste ($p < 0.001$)—had significant effects, as well as the interaction between them (p = 0.002). The evaluated toothpastes did not affect the NIR specimens. However, Bianco resulted in an increase in carbonate content for the IR specimens, while the other toothpastes led to similar reductions. Significant variations in carbonate content were primarily observed in IR and NIR specimens when Colgate was used.

For phosphate v1-v3, only irradiation (p = 0.011) and the interaction between irradiation and toothpaste (p = 0.002) sig-

Table 3. Mean (standard deviations) changes in roughness due to toothbrushing according to the specimens' irradiation and toothpaste used; values expressed as 10^{-2} µm.

Toothpastes	Groups	
	NIR	IR
Bianco	3.3 (1.5) Ba	5.5 (4.4) Aa
Sensodyne	3.5 (1.5) Ba	5.5 (4.0) Aa
Colgate	3.1 (2.0) Ba	4.0 (0.8) Aa

Distinct letters (uppercase comparing the specimens, lowercase comparing the toothpastes) indicate statistical differences at Tukey's test ($p < 0.05$).

Table 2. Means (standard deviations) of the specimens' surface roughness and components measured at baseline before the toothbrushing procedures.

Groups	Roughness Ra (10^{-2} µm)	FTIR analysis			
		Amide I (x10^{-1})	Phosphate v1-v3	Carbonate	Phosphate
NIR	3.7 (1.0)	1.0 (0.5)	11.8 (0.9)	1.8 (0.3)	4.5 (0.4)
IR	5.1 (1.2)	2.4 (2.2)	12.5 (3.0)	0.9 (0.5)	6.5 (2.1)
p-value*	< 0.001	< 0.001	0.213	< 0.001	< 0.001

*Calculated by *t*-test.

Table 4. Mean (standard deviations) changes in enamel components after the toothbrushing according to the specimens' irradiation and toothpaste used.

Enamel components	Toothpastes	Groups	
		NIR	IR
Amide ($x10^{-1}$)	Bianco	0.47 (0.54) ^{Aa}	-0.71 (0.85) ^{Ba}
	Sensodyne	-0.25 (0.35) ^{Ab}	-2.32 (3.20) ^{Bb}
	Colgate	-0.56 (0.47) ^{Ab}	-1.63 (1.05) ^{Bb}
Carbonate	Bianco	-0.21 (0.20) ^{Aa}	-0.51 (0.14) ^{Ab}
	Sensodyne	-0.16 (0.29) ^{Aa}	0.12 (0.45) ^{Aa}
	Colgate	-0.16 (0.32) ^{Aa}	0.53 (0.78) ^{Ba}
Phosphate V1-V3	Bianco	1.24 (1.10) ^{Aa}	-2.76 (2.18) ^{Bb}
	Sensodyne	0.97 (0.66) ^{Aa}	-2.18 (2.08) ^{Ab}
	Colgate	-0.19 (0.65) ^{Aa}	1.55 (5.70) ^{Aa}
Phosphate	Bianco	-0.24 (0.35) ^{Ba}	2.89 (1.23) ^{Aa}
	Sensodyne	-0.04 (0.65) ^{Aa}	0.99 (2.03) ^{Aa}
	Colgate	0.44 (0.61) ^{Aa}	-1.82 (3.05) ^{Bb}

For each enamel component, distinct letters (uppercase comparing the specimens, lowercase comparing the toothpastes) indicate statistical differences at Tukey's test ($p < 0.05$).

nificantly affected the results, while the toothpaste factor ($p = 0.181$) had no isolated effect. In IR specimens, Colgate increased phosphate v1-v3 values, whereas the other toothpastes resulted in similar losses. In contrast, there were no significant changes in phosphate v1-v3 levels for NIR specimens regardless of the toothpaste used.

Regarding phosphate content, only the toothpaste factor ($p < 0.001$) significantly influenced the results, while irradiation alone ($p = 0.132$) had no isolated effect. However, the interaction between irradiation and toothpaste was significant ($p < 0.001$). Irradiating the specimens did not affect changes in phosphate content when Sensodyne was used. In contrast, Bianco resulted in greater increases in phosphate content for IR specimens compared to NIR ones, while the opposite trend was observed with Colgate. For control specimens, Bianco led to the lowest phosphate content changes, with no significant differences among the other toothpastes. In NIR specimens, brushing with Colgate resulted in the highest phosphate content changes, with no differences noted among the other toothpastes.

Discussion

The findings of this study indicate that irradiation alters the effects of toothbrushing on the roughness and composition of enamel. The increase in enamel roughness was more pronounced in the IR specimens. In turn, the type of toothpaste used had no significant impact on roughness changes in both NIR and IR specimens. In contrast, the modifications to enamel composition resulting from toothbrushing were influenced by the interaction between irradiation and the toothpaste used. Therefore, the null hypothesis of the study was rejected.

The results of the present study indicate that solely irradiating enamel increased its roughness, with an average Ra value of 1.4×10^{-2} µm. This increase is nearly half of the roughness caused by toothbrushing, which ranged from 3.1 to 3.5 in the NIR specimens. Notably, the increased surface roughness in IR enamel appears to be closely related to compositional changes. FTIR analysis revealed that irradiation increased the contents of amide I and phosphate while decreasing carbonate content.

The rise in amide I levels can be attributed to the breakdown of chemical bonds within collagen induced by radiation.[20] Radiation causes the dissociation of water molecules, releasing peroxides that can reorganize and alter the chemical bonds of collagen.[11] This structural change results in alterations to the amide I band area, indicating reorganization and potential denaturation of collagen fibers.[21] Therefore, the increase in the amide I peak following radiotherapy underscores the significant impact of radiation on the structural integrity and organization of collagen.[16]

The phosphate bands v1 and v3, which represent the main mineral component of enamel, are predominantly found in the form of hydroxyapatite.[17] The incorporation of carbonate into the enamel structure is identified and quantified using the v3 carbonate bands at 1460 and 1425 cm^{-1}.[8] The decreased crystal clarity of carbonate and increased phosphate levels may result from planar carbonate ions replacing tetrahedral phosphate ions in the hydroxyapatite structure.[17] Furthermore, the tissue becomes more vulnerable to acids due to the reduction in carbonate ions, leading to an imbalance that modifies and accelerates the breakdown process of tooth structure.[17,21]

It has been demonstrated that ionizing radiation affects enamel, weakening its structure and increasing its susceptibility to degradation.[22] Notably, the increase in enamel surface roughness caused by toothbrushing was more pronounced in IR specimens, with no significant effect observed from the toothpaste used. The lack of differences among the toothpastes in both NIR and IR specimens can be attributed to their relatively low abrasiveness. Interestingly, despite their minimal effect on roughness, the toothpastes significantly impacted the enamel surface composition.

The combination of irradiation and different toothpastes either increased or decreased the levels of the analyzed structural components. These variations may stem from the distinct chemical compositions of each toothpaste. As observed in baseline measurements, irradiation alone increased the amide I content of enamel; however, the brushed IR specimens exhibited lower amide I levels than the brushed NIR specimens. A possible explanation for this discrepancy is that the increased abrasion (reflected in higher roughness changes) in IR specimens may lead to further loss of amide I. Interestingly, the use of Bianco toothpaste tended to increase the amide I content, suggesting that a specific component in this toothpaste may contribute to these findings.

The phosphate contents and the phosphate v3-v1 ratio tended to increase with irradiation at baseline (though the latter was not significant). While toothbrushing tended to result in higher phosphate levels for IR specimens, the opposite trend was observed for the phosphate v3-v1 content. Interestingly, the behavior of Colgate toothpaste differed from that of the other toothpastes. Additionally, lower carbonate content was observed in IR specimens at baseline, but toothbrushing generally increased carbonate levels in these specimens (except for Bianco). Given that the exact composition of these toothpastes and their precise interactions with tooth substrate are unknown, any explanations for the observed results tend to be speculative, highlighting the need for further studies.

To maintain the integrity of the enamel surface and avoid introducing uncontrolled variables, teeth should be washed and immersed in distilled water.[23, 24] Distilled water was chosen as the storage solution in this study because it facilitates radiolysis without significantly interacting with dental tissues.[24] This approach ensures that the study focuses on the direct effects of irradiation and toothpaste on enamel without introducing additional confounding factors.

Although in vitro studies offer valuable insights into enamel behavior under controlled conditions, their findings must be interpreted cautiously, as they cannot fully replicate the complex and dynamic environment of the oral cavity.[8,25] First, bovine enamel was used as a substitute for human enamel; while commonly employed in dental research due to its availability and comparable mineral content, structural and compositional differences may limit the direct extrapolation of findings to human teeth.[26] Second, although the irradiation protocol employed (total dose of 72 Gy administered in daily fractions of 1.8 Gy) is clinically relevant, it represents only one specific therapeutic scenario. Typically, radiotherapy for head and neck cancer follows protocols with total doses ranging from 50 to 72 Gy, administered in daily fractions of 1.8 to 2 Gy, five times per week over 5 to 7 weeks, depending on the prescribed total dose.[27] Therefore, variations in clinical protocols and advances in radiotherapy techniques may produce different effects on dental tissues.[28] Third, while surface roughness and chemical composition were assessed, additional analyses such as microhardness, bond strength, or morphological evaluations using scanning electron microscopy could provide further insights into the structural integrity and mechanical properties of irradiated enamel.[29] Finally, the brushing simulation was limited to a 30-day period, which may not fully capture the long-term cumulative effects of daily oral hygiene practices over extended periods, particularly in patients undergoing or recovering from radiotherapy. Future studies addressing these aspects are warranted to better understand the full scope of enamel alterations under clinical conditions.

The results revealed that radiation exposure and toothpaste significantly affect enamel composition and roughness. Clinically, increased enamel roughness and compositional changes can promote bacterial adhesion and biofilm accumulation, potentially leading to irreversible damage to dental tissues. Additionally, these alterations may contribute to gingival recession, dentin hypersensitivity, staining, microcrack formation, increased susceptibility to demineralization and caries, enamel fragility with risk of delamination and fractures, all of which can compromise both oral health and dental aesthetics.[8,30]

Future research is needed to investigate how different components of toothpastes affect enamel structures before and after irradiation, to understand their interactions better and optimize dental care strategies. Clinical studies are particularly necessary to validate these findings and to assess the efficacy of various oral care products in minimizing enamel degradation and preserving oral health in patients undergoing radiotherapy.

Conclusions

Irradiation significantly increased enamel roughness and altered the composition of enamel components, particularly amide I, carbonate, and phosphate. Toothbrushing further increased enamel surface roughness, which was more pronounced in irradiated specimens, regardless of the toothpaste used. In terms of compositional changes in enamel due to toothbrushing, these outcomes were dependent on both irradiation and the toothpaste applied.

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Conflict of interest

The authors have no conflicts of interest to declare.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

CRediT authorship contribution statement

Caroline Garcia Orsi: Investigation, Methodology, Formal analysis and Writing – original draft. **André Luis Faria-e-Silva:** Data curation, Formal analysis, Writing – review & editing. **Nayara Teixeira de Araújo Reis:** Investigation, Methodology, Formal analysis. **Tássio Edno Atanasio Pitorro:** Investigation, Methodology, Formal analysis. **Carlos José Soares:** Data curation, Formal analysis, Writing – review & editing. **Priscilla Barbosa Ferreira Soares:** Supervision, Conceptualization, Funding acquisition, Project administration, Resources and Writing – review & editing.

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Efeito da escovagem com diferentes pastas na rugosidade/composição do esmalte irradiado: estudo *in vitro*

RESUMO

Objetivo: Avaliar o efeito da escovagem com diferentes pastas dentífricas na rugosidade e na composição mineral do esmalte irradiado e não irradiado.

Métodos: Cento e vinte coroas bovinas foram distribuídas aleatoriamente entre três grupos irradiados e três não irradiados sujeitos a escovagem com Bianco Pro Clinical 1% TCP, Colgate Sensitive Pro-Relief ou Sensodyne Sensitivity & Gum (n=20). A irradiação foi realizada em doses fracionadas, totalizando 72 Gy. As amostras foram escovadas num ciclo de simulação de 30 dias. A rugosidade (Ra-µm) e a composição química foram medidas antes e depois da escovagem. Os dados foram analisados usando o teste ANOVA de duas vias, seguido do teste de Tukey, com um nível de significância de α=0,05.

Resultados: O esmalte irradiado apresentou maior rugosidade do que o esmalte não irradiado mesmo antes da escovagem e apresentou níveis maiores de amida I e fosfato, mas menores de carbonato. Após a escovagem, a rugosidade aumentou no esmalte irradiado, independentemente da pasta dentífrica utilizada. As alterações na composição do esmalte pós-escovagem variaram conforme a pasta dentífrica. Sensodyne e Colgate causaram maiores reduções em amida I do que Bianco. O esmalte irradiado tratado com Bianco mostrou um aumento no conteúdo de carbonato, enquanto aqueles escovados com Colgate apresentaram níveis mais altos de fosfato V1-V3.

Conclusões: A irradiação aumentou a rugosidade e a escovagem amplificou esse efeito, sem diferenças significativas entre as pastas dentífricas avaliadas. Tanto a irradiação como a escovagem influenciaram a composição química do esmalte, com alterações variáveis de acordo com a pasta dentífrica específica. (Rev Port Estomatol Med Dent Cir Maxilofac. 2025;66(3):108-114)

Palavras-chave:
Cuidados dentários
Radioterapia
Escovagem dentária