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Effect of gamma radiation on human enamel chemical and physical properties

Pollyanna Nogueira Ferreira da Silva¹, Maria Carolina Fernandes Barcellos², Fernanda Calvo Costa², Célio dos Santos Silva², Sílvio Manea³, Odair Lellis Gonzalez⁴, Vitor Ribeiro Jardim⁵, Gislene Valdete Martins³, Nelson Batista Lima⁶, Anelyse Arata Found⁷, Grace Mendonça De Souza⁸, Estela Kaminagakura⁹ and Rubens Nisie Tango^{9*}

Abstract

Background Gamma radiation is still used in developing countries for head and neck cancer treatment. Irradiated dental enamel undergoes dose-dependent chemical and mechanical changes that can hinder oral rehabilitation. Understanding these processes can be useful to professionals to plan safer long-lasting treatments. This study aimed to evaluate different doses of gamma radiation on the chemical and mechanical properties of human dental enamel.

Methods Sixty human third molars were divided into six groups ($n = 10$), according to dose: 0; 20; 40; 50; 60; or 70 Gy, in daily increments of 2 Gy. After irradiation, the composition and carbonate/ phosphate ratio (C: P) were evaluated by Fourier Transformed Infrared Spectroscopy (FTIR). X-ray diffraction (XRD) was used to evaluate crystal size (shape factor) and crystallinity. Hardness, elastic modulus, and scratch resistance were measured, and the microstructure was observed by scanning electron microscopy (SEM). Data of C: P, crystallinity (%), shape factor (nm), hardness (VHN - Vickers Hardness Number), and elastic modulus (GPa - Gigapascals) were submitted to analysis of variance and to Tukey's test ($\alpha = 0.05$).

Results FTIR showed carboxylic acid in the irradiated groups and an increase in C: P for the 70 Gy group ($p = 0.015$), but with no change in crystalline structure. A significant decrease was observed in the values of hardness ($p = 0.0000$) and the elastic modulus ($p = 0.0000$) in the irradiated groups. For scratch test, the 60 and 70 Gy groups showed lower values for initial spallation. SEM showed spaced rounded peaks of enamel prisms in the 60 and 70 Gy groups.

Conclusions Gamma radiation negatively affected the chemical and physical properties of human enamel.

Trial registration Plataforma Brasil, CAAE: 66495417.1.0000.007.

Keywords Dental enamel, Radiotherapy, Chemical properties, Mechanical properties

*Correspondence:
Rubens Nisie Tango
rn.tango@unesp.br

Full list of author information is available at the end of the article



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Background

Oral cancer is ranked as the eighth most prevalent neoplasm globally, with an incidence ranging from 1 to 10 cases per 100,000 individuals [1], and approximately 300,000 new cases arising each year [2]. Radiation therapy stands as a viable treatment option [3], hence there is the preservation of anatomical structures, potential regression of the disease, reduction in tumor volume, and prevention of relapses and metastasis [4].

The mechanism of radiotherapy is based on ionizing radiation to either interact with the cellular DNA of the cancerous cell, inducing cell death (apoptosis) and consequent reduction in lesion volume, or the radiolysis of water to generate new chemical bonds toxic to the cells [4]. Usually, radiotherapy with gamma radiation is divided into daily doses of 2 Grays (Gy) for 5 days per week during approximately 7 weeks, resulting in a total delivered dose of 50–70 Gy [4–6]. However, this treatment focused on cancer remission may yield undesired outcomes such as changes in dental structure, osteoradionecrosis, xerostomia, and mucositis [7–10].

Studies evaluating the effect of gamma radiotherapy on chemical composition, microstructure and mechanical properties of human enamel are controversial. Some report no effect on enamel chemical and microstructural composition [11], while others report significant alterations on dental enamel hardness and toughness [12–19]. Delamination is one of the alterations on enamel, which may lead to dentin exposure in the oral environment [17]. Due to its higher organic and lower inorganic content compared to enamel [19–21] dentin is more prone to demineralization process [22], which is related to higher risk of tooth loss.

The aim of this study was to evaluate the effect of gamma irradiation on chemical and mechanical properties of human dental enamel. The null hypotheses were that chemical composition, crystalline structure, and mechanical properties (hardness, elastic modulus, and scratch resistance) of human dental enamel would not be modified upon different doses of ionizing radiation.

Methods

Sixty human third molars, extracted by orthodontic reasons were cleaned and stored frozen in 2% chloramine for up to 6 months after extraction. The research project was submitted to the Institutional Ethics Comitee, following national regulations of the National Council of Health - Plataforma Brasil in accordance with the Declaration 105 of Helsinki, and approved by the Ethics Committee Plataforma Brasil, which deemed the consent to participate unnecessary. Ethics approval was obtained - CAAE: 66495417.1.0000.007. Teeth were sectioned in a buccal-lingual and a mesio-distal direction with an Extec High Concentration diamond blade (Extec) mounted in

a cutting machine (IsoMet 1000 Precision Saw, Buehler) under water cooling, to generate enamel blocks (crown quarter). The specimens (blocks) were randomly (www.random.org) allocated into groups according to the radiation dose, attempting to the fact that each group should present equative number of blocks from different quarters ($n=10$): control (0 Gy), 20, 40, 50, 60, and 70 Gy. Gamma irradiation was delivered with a ^{60}Co radiotherapy device (Eldorado 78, Atomic Energy of Canadian) with a fractionated dose of 2 Gy per day, 5 days per week, for as long as required for each experimental group. Radiation dose was verified with a dosimeter (Radiation Monitor Controller, Model 2026 C). Specimens were stored in distilled water at 37 °C between each session and water excess was dried softly for all analysis.

Analysis of chemical composition

Enamel composition was analyzed at baseline and upon irradiation using a spectrophotometer (Perkin Elmer Spectrum One Fourier Transform Infrared Spectrometer), with the following arrangement: transmission range between 4000–400 cm^{-1} , interval of 2 cm^{-1} , and speed of 0.6329 $\text{cm}^{-1}/\text{sec}$. After correction of the baseline and normalization, the relationship between the integrated areas of the chemical compounds CO_3^{2-} ν_2 was performed (band between 810 and 850 cm^{-1}) and PO_4^{3-} ν_1 , ν_3 (band between 885 and 1090 cm^{-1}), which resulted in the carbonate/phosphate ratio (C: P) [12].

Analysis of crystallinity, crystallite size, and shape factor

The amorphization (%) and crystallographic changes induced by ionizing radiation was measured by X-ray diffractometry (XRD) (Panalytical, model X'Pert Powder). Five specimens per group and dental enamel powder (hydroxyapatite) were submitted to $\text{Cu-K}\alpha$ radiation for identification of amorphization induction (scanning between 5° and 80°, angular pitch 0.02°, speed 10 s/ per step), and determination of the mean crystallite size (angular pitch of 0.01°, speed 40 s/step, peaks of 002 - between 25° and 28°, 202 - between 32° and 36° and 213 - between 48° and 51°). Bruker-AXS Diffrac EVA software (Bruker) was used for the calculation of the phase fraction by the following equation:

$$\% \text{ amorphous phase} = \frac{\text{diffracted intensity of amorphous phase}}{\text{total diffracted intensity}}$$

Since the crystallite values vary according to the crystallographic plane, it was assumed that they presented an elliptical format [23]. For the determination of the major and minor axis of the ellipse, a graph was plotted considering the angles between planes 002 and 202 = 40.11°, and 002 and 213 = 55°. The Average Crystal Size (ACS) value of each plane and plotting of ellipse Cartesian

coordinates were calculated (Origin 6.0 software). The ellipse was plotted to determine the larger (P1) and the smaller (P2) crystals half-axis, which were used to calculate the shape factor. Scherrer’s formula was used to calculate crystal size (nm) [12]:

$$D = \frac{0,89 \lambda}{\beta \cos \theta}$$

where D is the crystal size (nm), λ is the wavelength (CuK α), β is the width at half height of the hydroxyapatite peaks (002, 202, 213), and θ is the diffraction angle.

Scanning field electron microscopy (Mira 3, Tescan) was used to analyze the enamel surface of representative specimen of each group. The specimens were gold-sputtered (SC7620 ‘Mini’ Sputter Coater/Glow Discharge System, EMITECH) and were analyzed under 5 kV, 5.0 spot and magnification of 2000x, 5000x and 20000x.

Hardness and elastic modulus

Specimens of each group (n=10) were sequentially polished with # 1200, 2400 and 4000 grit silicon carbide paper in a rotary polisher under water cooling and were cleaned in distilled water in an ultrasonic bath (Cristófoli Ultrasonic Washer) for 5 min. A Berkovich indenter coupled to a nanoindentation tester (NHT2, Anton Paar) was used to perform 3 loading/partial unloading cycles (10 Hz resonance frequency, 25 mN load, 10 s) to measure hardness (VHN) and elastic modulus (GPa) [24].

Scratch resistance test

Specimens (n=3) were submitted to scratch resistance test in a Micro Scratch Tester (CSM- Instrumental - Antoon Paar) with a Rockwell 1 μ m radius diamond indenter (progressive load from 0.1 N to 15 N, speed of 0.5 mm/min, and 1.5 mm travelling length).

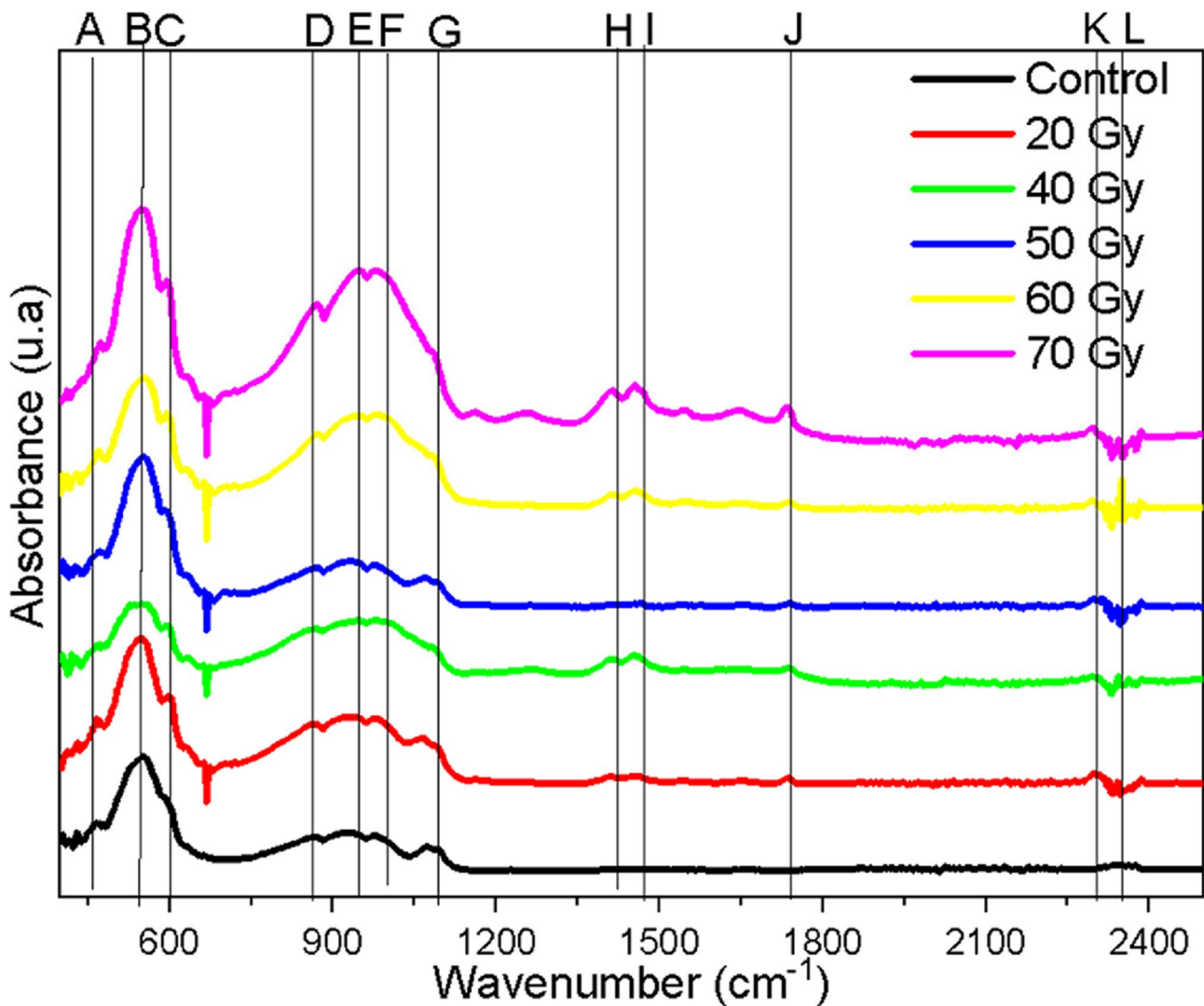


Fig. 1 FTIR spectra of dental enamel after radiation

Data analysis

Two-way analysis of variance (ANOVA) and Tukey test ($\alpha = 0.05$) were used (Statistix 8.0" software) to analyze C: P data, while one-way ANOVA and Tukey test were used for crystallinity, crystal size and hardness comparisons.

Results

Figure 1 shows the vibration spectra (FTIR) of the enamel fragments submitted to different doses of irradiation and the letters represent a set of bands of the assignments presented in Table 1.

It can be suggested that bands A, B, C, E, F, and G refer to the chemical element phosphate (PO_4), while the bands D, H and I are allocated to the carbonate group (CO_3). Additionally, formation of carboxylic acid ($C=O$), represented in the bands J, K, and L [25, 26] was observed in irradiated groups. According to the results of 2-way ANOVA, treatment ($p = 0.005$) and irradiation dose ($p = 0.001$) affected the C: P (Table 2).

According to the results of one-way ANOVA, irradiation did not affect the crystallinity and shape factor of enamel ($p = 0.48$) (Table 3; Fig. 2) but increased the carbonate content and decrease of phosphate concentration in enamel, because of amorphization of enamel prism (Fig. 3).

According to the results, the hardness and elastic modulus were affected by irradiation dose ($p = 0.0000$), and the lowest hardness value was found in the 70 Gy group (Table 4). Specimens exposed to 40 Gy or more, presented lower values of elastic modulus compared to non-irradiated specimens.

Results indicated that doses of 60 Gy and 70 Gy decreased scratch resistance compared to another groups (Fig. 4; Table 5). Figure 4 shows the tracks performed in one sample per experimental group; it is possible to observe the entire extension of the scratch, initial cracks, and spallation. Because of decreased scratch resistance,

Table 1 Assignment table of the band sets in figure 1

Frequencies (cm^{-1})	Attribution	Set of bands	References
≈ 500	PO_4^{-3}	A	1,2
543	PO_4^{-3}	B	1,2
599	PO_4^{-3}	C	1,2
868	Carbonate	D	1,2
928	PO_4^{-3}	E	1,2
980	PO_4^{-3}	F	1,2
1084	PO_4^{-3}	G	1,2
1445	Carbonate	H	1,2
1460	Carbonate	I	1,2
1696	Carbonyl ($C=O$)	J	2
2300	Carboxylic Acid	K	2
2390		L	2

Key references: (1) Reyes-Gasga et al., 2013; (2) Vargas-Becerril et al., 2018

Table 2 Results for carbonate/phosphate ratio data (Mean ± SD) and Tukey HSD test results

Dose	Before	After
20 Gy	0.31 (0.02) ^{a,AB}	0.32 (0.01) ^{a,A}
40 Gy	0.29 (0.02) ^{a,B}	0.33 (0.02) ^{a,A}
50 Gy	0.31 (0.01) ^{a,AB}	0.32 (0.03) ^{a,A}
60 Gy	0.34 (0.01) ^{a,A}	0.35 (0.02) ^{a,A}
70 Gy	0.30 (0.02) ^{b,AB}	0.34 (0.01) ^{a,A}

Different lowercase letters in each row ($p = 0.001$) and uppercase letter in each column represent significant differences ($p = 0.005$). Significance level = 5%

Table 3 Tukey HSD test result for crystallinity and shape factor data, according to the dose

Dose	Crystallinity(%)	Shape Factor
Controle (0 Gy)	70.92 (3.39) ^A	2.31 (0.24) ^a
20 Gy	70.72 (2.45) ^A	2.05 (0.35) ^a
40 Gy	65.90 (3.03) ^A	1.92 (0.47) ^a
50 Gy	68.14 (3.13) ^A	2.24 (0.36) ^a
60 Gy	69.24 (4.96) ^A	2.30 (0.44) ^a
70 Gy	68.04 (1.64) ^A	2.19 (0.24) ^a

Different lowercase letters within the same column represent significant differences ($p = 0.49$)

70 Gy group showed an ejection of enamel outside the track (trackside delamination) (Fig. 4D) and lower value of spallation starting (8.7 ± 0.9) (Table 5).

Discussion

The chemical composition and mechanical properties were affected by gamma irradiation doses, despite of no modification of the crystalline structure. Thus, the null hypothesis were rejected. Furthermore, it modified the chemical composition of the dental enamel, generating carboxylic acid and oxidation of water molecules in hydroxyl and hydrogen ions, which was confirmed by the

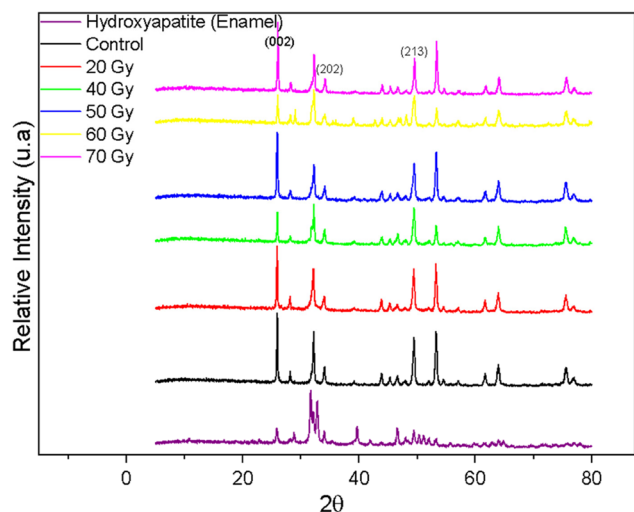


Fig. 2 X-ray diffraction (XRD) of dental enamel samples

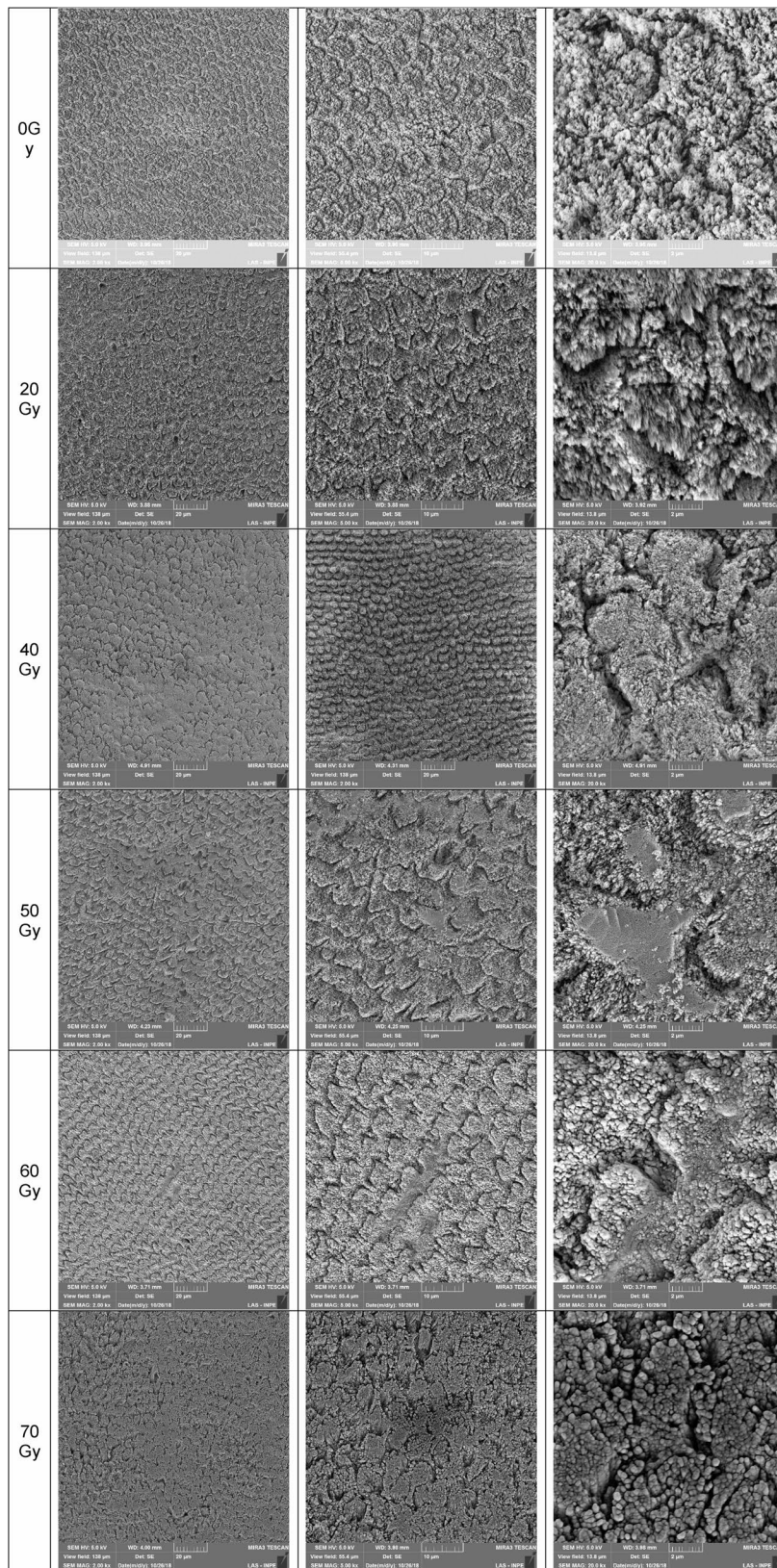


Fig. 3 Surface of enamel prisms exposed to different levels of irradiation. More rounded and spaced enamel rod prisms were observed under high magnification on irradiated groups

Table 4 Results of surface hardness (VHN) and modulus (GPa) as a function of irradiation dose

Dose	Hardness	Elastic modulus
Controle (0 Gy)	515.60 (56.20) ^A	107.67 (8.19) ^a
20 Gy	475.50 (40.96) ^B	105.27 (6.70) ^{ab}
40 Gy	464.07 (45.82) ^B	101.79 (4.05) ^{bc}
50 Gy	478.98 (31.29) ^B	100.11 (4.75) ^c
60 Gy	471.57 (23.76) ^B	100.65 (8.27) ^{bc}
70 Gy	465.48 (26.78) ^B	100.57 (5.83) ^{bc}

Different letters within the same column show significant differences ($p=0.000$) according to Tukey HSD results

presence of a carbonyl band (band J, K, L) in the irradiated specimens (Fig. 1). It was suggested that the degradation of the amide leads to formation of carbonyl groups [13, 25–28] due to the calcium-mediated electrostatic bonding break of the carboxylate chain side collagen groups to the phosphoapatite groups, as occurs when exposed to X-rays [29]. This degradation of both, proteins (amide) [28] and collagen types IV and VII [27] has as consequence the instability of dentin-enamel junction and the decrease of bond strength between enamel apatite crystals and the organic matrix. As result, dental enamel is more prone to fracture during mastication [13, 18, 27, 31].

The decrease of collagen types IV and VII in enamel is attributed to the direct radiolysis catalyzed by matrix metalloproteinase (MMP 20) [14, 27]. The denaturation of the organic matrix (Figs. 1 and 3; Table 1) may lead to gap formation in the dentin-enamel junction [18, 32], which under masticatory stress [12, 31] may open space for potential bacterial colonization and caries development [7–9].

C: P showed to be increased with 60 Gy and 70 Gy (Fig. 1 and in Table 2). This modification has been referred as a crystalline alteration, because the organic matrix interactions between enamel prisms, generating smaller crystallites [11, 12]. In SEM photomicrographs, it was seen as rounded hydroxyapatite rods in enamel prisms of irradiated specimens (Fig. 3). Although there was a change in the C: P (Fig. 1 and in Table 2) for 60 Gy and 70 Gy, indicating decrease of the mineral content of the enamel, no changes in crystallinity, crystallite size and shape factor were observed (Table 3). Thus, the null hypothesis that the ionizing radiation would not influence on the characteristics of the enamel crystal was accepted. On contrary, Qing et al., in 2015, reported alterations in enamel crystallinity and crystallite size using XRD. As far it was found in literature, there is no report of shape factor of the dental enamel. Rounded and spaced enamel rod prisms observed with 70 Gy can be considered as an interesting finding (Fig. 3).

XRD spectra (Fig. 2) shown changes in spectral patterns between groups. However, it can be due to enamel prisms orientation for anatomical shaping of tooth. Moreover, in Fig. 2; Table 3, it can be observed that dental enamel specimens were composed by hydroxyapatite despite the preferential crystallographic orientation of the plane (002). Although, in the shape factor (Table 3; Fig. 2) no changes in surface of enamel prisms were found upon exposure to different doses, corroborating with Barros da Cunha et al., in 2017 [11], but divergent from de data found by Duruk, Acar and Temelli, in 2020 [20] and also with Yao et al., 2024 [33]. It was possible to observe modification on enamel prism form after irradiation with 70 Gy (Fig. 3).

Results of the present study indicated that ionizing radiation has a negative effect on the hardness of human enamel. For elastic modulus, decrease of values were observed with exposure doses higher than 20 Gy (Table 4), what is in accordance with previous reports [11, 12, 15, 17, 18]. Therefore, the null hypothesis that radiation would not change mechanical properties of enamel was partially accepted. The decrease of tooth enamel hardness values seems to be caused by changes in microstructure and chemical composition after radiation.

The decrease of hardness values and the elastic modulus of the dental enamel caused by radiotherapy leads to a series of deleterious effects such as structural weakening and the possibility of enamel fracture [18, 27], clinically exposing the underlying dentin. The exposure of the dentin substrate results in a significantly higher susceptibility to caries lesions [25]. Other outcomes are expected as a consequence of radiotherapy, such as alteration of oral microflora, hyposalivation, the need of a softer diet, mucositis and burning mouth syndrome [5, 10, 30, 34], and these factors altogether encounter for even higher caries risk. Third molars were selected for the study because they were easiest to collect and because they would present higher composition homogeneity compared to other tooth in service in mouth.

It was verified that with exposures of 60 Gy and 70 Gy, there was a decrease in scratch resistance (Table 5), in accordance with Qing et al., in 2015. The failures in the scratch track (Fig. 4) were “ARC tensile” and “recovery spallation”. “ARC tensile” is a failure caused by compression stresses [35] and “recovery spallation” is the failure associated with inefficient elastic recovery along the track path causing plastic deformation of the substrate, which continued with residual tension generating off the track cracking [35]. In Fig. 4, “trackside delamination”, correlated to a lower resistance to scratching [35–38] was observed in the representative specimen of 70 Gy, which

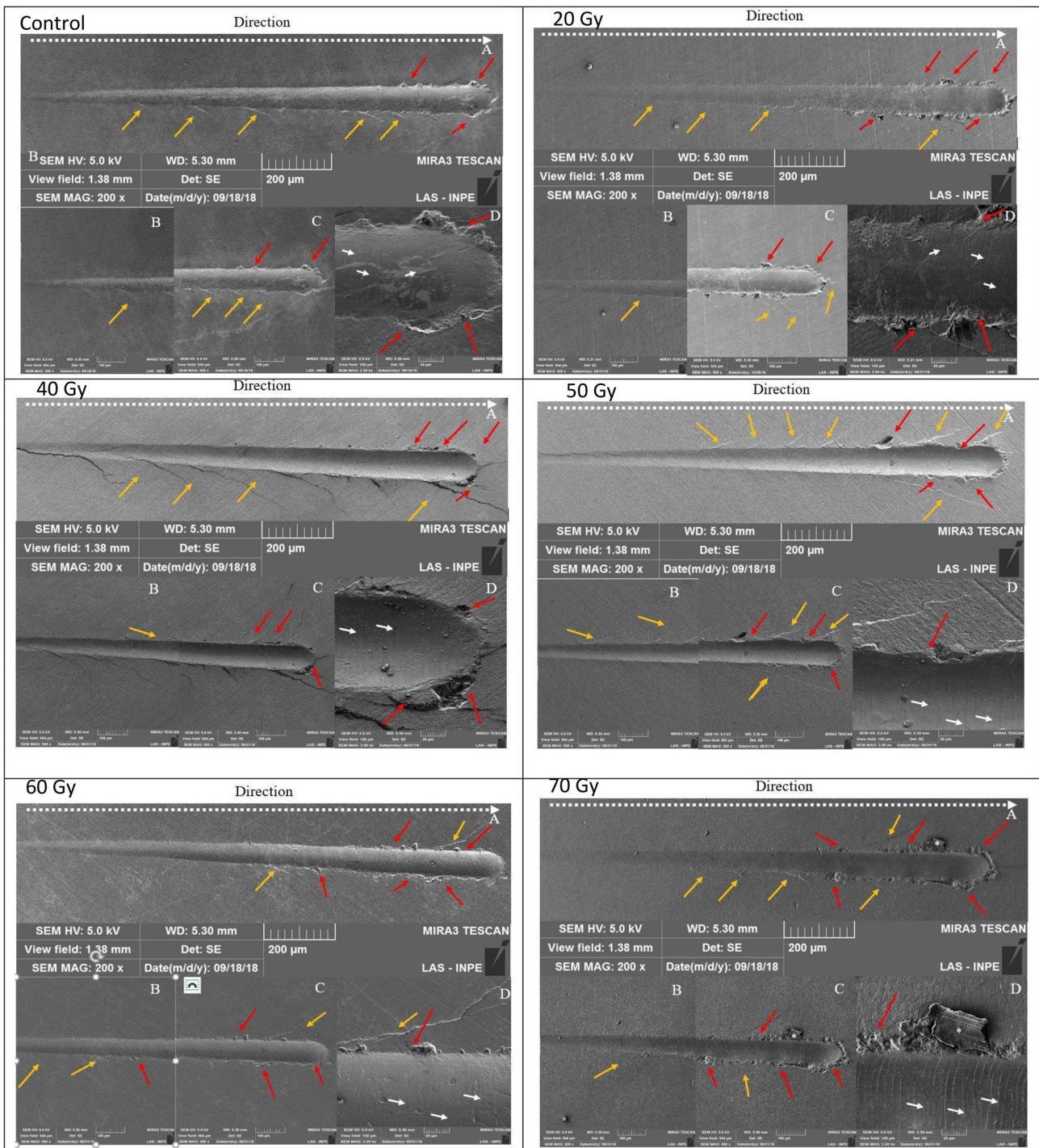


Fig. 4 Micrographs of the enamel surfaces submitted to scratch test. **A** Panoramic Scratch Map; **B**) Initial cracks; **C**) Initial Spallation; **D**) Recovery Spallation. Yellow arrows indicate lateral cracks, red arrows indicate the spallation, the white arrows indicate the Tensile ARC, the dotted arrow demonstrates the progressive loading direction of the track, and the asterisk indicates trackside delamination

showed an ejection of dental enamel. It can be emphasized that the load used in this study was not sufficient for any removal or detachment of enamel, exposing dentin. Extrapolation to clinical *in vivo* implications should be seen with care. However, it can be supposed that in

oral environment, where there is association of multiple challenging factors, such as mechanical and thermal stresses, toothbrush attrition, and acid challenge, enamel with trackside delamination would be prone to detachment exposing the underlying dentin.

Table 5 Values of critical load (N) for key events after the scratch test as a function

Group	Initial Cracks (\pm SD)	Initial Spallation (\pm SD)
Control	3.6 (1.3)	10.5 (0.3)
20 Gy	4.3 (0.2)	10.7 (0.3)
40 Gy	3.3 (0.1)	10.2 (0.2)
50 Gy	2.8 (0.2)	9.9 (0.5)
60 Gy	2.8 (0.3)	8.5 (0.6)
70 Gy	3.2 (0.5)	8.7 (0.9)

The results are shown as a function of irradiation dose

Conclusions

Limited by the methodology of this study, it was concluded that the hardness and elastic modulus of the dental enamel exposed to simulated therapeutic gamma radiation decreased by about 8.73% and 6.54%, respectively. The scratch resistance decreased especially in groups submitted to 60 Gy and 70 Gy. The irradiation up to 70 Gy provoked modification of C: P, but did not modify the crystallinity, crystallite size and shape factor of human enamel.

Abbreviations

GY	Grays
C: P	Carbonate/phosphate ratio
FTIR	Fourier Transformed Infrared Spectroscopy
XRD	X-ray diffraction
ACS	Average Crystal Size
VHN	Vickers Hardness Number
GPA	Gigapascals
MMP 20	Matrix Metalloproteinase-20
SEM	Scanning Electron Microscopy

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None.

Authors' contributions

PNFS acquired data, played an important role in interpreting the results and approved the final version; MCFB acquired data and approved the final version; FCC acquired data and approved the final version; CSS acquired data and approved the final version; SM acquired data and approved the final version; OLG played an important role in interpreting the results, drafted and revised the manuscript and approved the final version; VRJ acquired data and approved the final version; GVM acquired data, played an important role in interpreting the results and approved the final version; NBL played an important role in interpreting the results, drafted and revised the manuscript and approved the final version; AAF played an important role in interpreting the results, drafted and revised the manuscript and approved the final version; GMS played an important role in interpreting the results, drafted and revised the manuscript and approved the final version; EK played an important role in interpreting the results, drafted and revised the manuscript and approved the final version; RNT conceived and designed the work that led to the submission, acquired data, played an important role in interpreting the results, drafted and revised the manuscript and approved the final version.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the declaration 105 of Helsinki, and approved by the ethics committee plataforma brasil, which deemed the consent to participate unnecessary. Ethics approval was obtained - CAAE: 66495417.1.0000.007.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Private office, Sao Jose dos Campos, SP, Brazil

²Institute of Science and Technology, Sao Paulo State University - UNESP, Av. Eng. Francisco Jose Longo, 777, Sao Jose dos Campos, SP 12245-000, Brazil

³Space Engineering and Technology, National Institute for Space Research - INPE, Av. dos Astronautas, 1758, Sao Jose dos Campos, SP 12227-010, Brazil

⁴Department of Science and Aerospace Technology, Institute of Advanced Studies - IEAv/Brazilian Air Force, Trevo Cel. Av. Jose Alberto Albano do Amarante, 01, Sao Jose dos Campos, SP 12228-001, Brazil

⁵Department of Lasers and Applications, Institute of Advanced Studies - IEAv/Brazilian Air Force, Trevo Cel. Av. Jose Alberto Albano do Amarante, 01, Sao Jose dos Campos, SP 12228-001, Brazil

⁶Department of Materials Science and Technology, Nuclear and Energy Research Institute IPEN/CNEN-SP, Av. Prof. Lineu Prestes, 2241, Sao Paulo, SP 05508-000, Brazil

⁷Schulich School of Medicine & Dentistry, Western University, London, ON N6A 5C1, Canada

⁸School of Dentistry, University of Louisville, 501 South Preston Street, Louisville 40202, KY, USA

⁹Institute of Science and Technology, Sao Paulo State University - UNESP, Av. Eng. Francisco Jose Longo, 777, Sao Jose dos Campos, SP 12245-000, Brazil

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