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Scientific Article

Effect of Cumulative Ionizing Radiation on Flexural Strength, Flexural Modulus, and Elasticity Modulus of Dentin in Unerupted Human Third Molars

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Abstract

Purpose: This in vitro study aimed to investigate the changes in mechanical properties in dentin of third molars after radiation therapy using variable doses and frequencies.

Methods and Materials: Rectangular cross sectioned dentin hemisections (N = 60, n = 15 per group; >7 × 4 × 1.2 mm) were prepared using extracted third molars. After cleansing and storage in artificial saliva, random distribution was performed to 2 irradiation settings, namely AB or CD (A, 30 single doses of irradiation [2 Gy each] for 6 weeks; B, control group of A; C, 3 single doses of irradiation [9 Gy each]; and D, control group of C). Various parameters (fracture strength/maximal force, flexural strength, and elasticity modulus) were assessed using a universal Testing Machine (ZwickRoell). The effect of irradiation on dentin morphology was evaluated by histology, scanning electron microscopy, and immunohistochemistry. Statistical analysis was performed using 2-way analysis of variance and paired and unpaired *t* tests at a significance level of 5%.

Results: Significance could be found considering the maximal force applied to failure when the irradiated groups were compared with their control groups (A/B, $P < .0001$; C/D, $P = .008$). Flexural strength was significantly higher in the irradiated group A compared with control group B ($P < .001$) and for the irradiated groups A and C ($P = .022$) compared with each other. Cumulative radiation with low irradiation doses (30 single doses; 2 Gy) and single irradiation with high doses (3 single doses; 9 Gy) make the tooth substance more prone to fracture, lowering the maximal force. The flexural strength decreases when cumulative irradiation is applied, but not after single irradiation. The elasticity modulus showed no alteration after irradiation treatment.

Conclusions: Irradiation therapy affects the prospective adhesion of dentin and the bond strength of future restorations, potentially leading to an increased risk of tooth fracture and retention loss in dental reconstructions.

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Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

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Introduction

Radiation therapy is a treatment for head and neck cancer that uses ionizing irradiation[1] and is commonly used as a primary adjuvant therapy to surgical treatment in conjunction with chemotherapy or as a palliative treatment for advanced or inoperable tumors.[2] The oral cavity manifestations of gamma irradiation can include not only xerostomia, mucositis, candidiasis, dysgeusia, loss of taste, muscle trismus, vascular changes, and osteoradionecrosis but also a possible contribution to an increased risk of irradiation tooth decay.[3,4] Patients undergoing radiation therapy have an increased risk of developing irradiation caries throughout their life, not only during or immediately after treatment.[5,6] Irradiation caries is caused by indirect and direct effects. Indirect effects include changes in salivary flow rate and saliva quality, difficulty in performing adequate oral hygiene, adoption of a soft diet due to difficult swallowing, and microbiota shift.[6-8] Direct effects on the tooth include changes in the crystalline structure, dentinoenamel junction, acid solubility of enamel, and microhardness caused by alteration in the organic matrix.[2,5,6]

The enamel of nonirradiated teeth presents organized prisms with transverse and oblique arrangement surrounded by interprismatic portions. The prismatic structure remains unaltered after irradiation, while the interprismatic region becomes more evident.[4] It has been reported that irradiation affects the organic matrix of enamel.[9,10] Free radicals and reactive oxygen species that react with water in the interprismatic region are considered to contribute to the changes. The dentin of nonirradiated teeth presents well-defined dentinal tubules and an organized collagen fiber network. Morphologic alterations manifest after 30 and 60 Gy irradiation doses in the intertubular, peritubular, and intratubular dentin. At 30 Gy, fissures in dentinal structure become evident, while at 60 Gy, the dentinal tubules become obliterated. The collagen fibers gradually fragment with the increase of irradiation doses.[4] Free radicals negatively affect the hydration of collagen fibers if the irradiation causes alteration in the secondary and tertiary structures of proteins. Degradation of the collagen fibers network and obliteration and fissures in the dentinal structure are a result of loss of collagen fibers hydration.[11] Increasing the dose results in progressive micromorphological alterations of both enamel and dentin structures. While the microhardness of the permanent teeth increases after irradiation, the values for the microhardness of the enamel in the superficial depth decrease up to a cumulative dose of 30 Gy but increase at higher doses, with the middle and deep enamel not differing from nonirradiated enamel. The superficial and deep dentin microhardness has no alteration compared with the nonirradiated dentin, while the middle dentin microhardness decreases significantly. Overall, the microhardness of dentin decreases after every 10-Gy cumulative dose from 10 up to 60 Gy.[4]

A possible explanation could be that dentin has a higher water content than enamel (10% vs 4% by weight). Hence, tissue with higher water content could be more vulnerable to the radiation effects and have stronger effects on mechanical properties of tissues.[12] As dentin supports enamel, a softer dentin tissue becomes less efficient, allowing the occurrence of fractures and cracks in the enamel.[13] The higher microhardness of the superficial layer of enamel turns it more friable and susceptible to crack formation, possibly contributing to dentinal hypersensitivity and favoring marginal infiltration of restorations.[4] The degradation of the organic portion of dentin could also interfere with the adhesion of resinous restorative materials.[13] Several studies have focused on the visualization and interpretation of consequences of radiation therapy on the macromorphological structure changes in human permanent teeth but did not perform mechanical property measurements such as flexural strength, flexural modulus, or elasticity parameters.[4,5] It has been reported that mechanical properties of dental tissue change after radiation therapy and consequently can affect the outcome of restorative dental treatment of patients with head and neck cancer undergoing radiation therapy.[4] Therefore, the objectives of this study were to investigate the effect of cumulative ionizing radiation on mechanical properties of the dentin and to show structural and morphologic alterations. The null hypothesis tested was that radiation doses would not show significant differences on the mechanical properties of dentin.

Methods and Materials

Pre-experimental procedures

Written informed consent for research purpose of the extracted teeth was obtained by all donors prior to extraction according to the directives set by the National Federal Council. Ethical guidelines were strictly followed and irreversible anonymization was performed in accordance with State and Federal Law (World Medical Association, Declaration of Helsinki, 2013; Human Research Act, 2015).[14,15]

Specimen preparation

One rooted maxillary and mandibular unerupted third molars (n = 30) were selected, stored (in distilled water

and thymol solution at 4°C to inhibit microbial growth), and used 3 weeks after extraction. The apical third of the root was embedded in epoxy resin/acrylic blocks using a conventional composite (FiltekTM Supreme XTE Flowable Composite, 3M ESPE, St Paul, MN) to stabilize the tooth for the cutting procedure.

Thereafter, the tooth crown was cut off 1 mm below the cementoenamel junction, and dentin specimens with a rectangular cross section (>7 × 4 × 1.2 mm) were cut in mesiodistal direction into buccal and palatinal/lingual halves of the teeth using an electrical precision diamond wire saw with blade diameter of 0.17 mm and 30-μm roughness under constant water cooling (Well, Walter Ebner, Locle, Switzerland). After cutting, they were polished manually under water flow with 1200 grit silicon carbide paper (Streuers, Willich, Germany) until a flat surface was obtained. The thickness was verified using a digital micrometer (Mitutoyo, Kamagawa, Japan). Finally, they were washed in running water, dried with gauze, ultrasonically cleaned in water for 5 minutes, and placed in 12-well acrylic cell culture plates filled with artificial saliva, which was prepared according to the chemical components (chemical compounds of artificial saliva stock solution (sodium bicarbonate 2.4 g, potassium chloride 1.7 g, magnesium chloride 0.1 g, calcium chloride 0.2 g, potassium thiocyanate 0.2 g, potassium dihydrogen phosphate 0.7 g, boric oxide 0.1 g, double-distilled water 1000 mL) and artificial saliva (sodium bicarbonate 1.62%, 51.5 mL; stock solution 2.4 g/L, 198 mL; double-distilled water 198 mL; Natrosol HR 2.5 g; glycerin 85%, 50 g). The tooth sections were obtained to perform 3-point bending tests, scanning electron microscopy (SEM), and immunohistochemical evaluation.

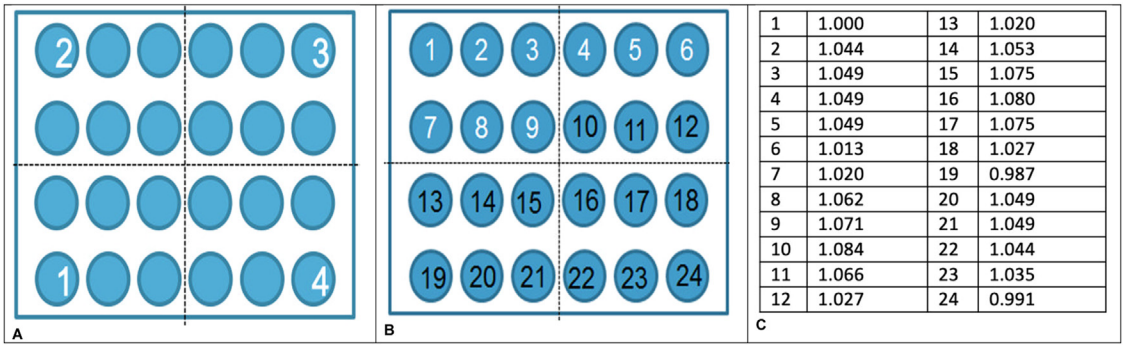
Experimental design/radiation procedure

Both hemisections of each tooth were randomly distributed to either the first 2 groups (A and B) or the last 2 groups (C and D). Groups A and C were irradiated with a cumulative dose of 60 Gy varying in sequences and single doses. While group A was irradiated with a dose of 2 Gy per fraction (1 fraction per day, 5 times a week) on a 6-week course, group C was irradiated with 9 Gy per fraction (1 fraction per day, 3 times; total dose = 60 Gy). The total dose in both groups was 60 Gy. Groups B and D served as nonirradiated control groups of groups A and C, respectively. During the radiation process the specimens were stored in 1 mL sodium chloride in the outer 16 wells of the 24-well plate to minimize the radiation inaccuracy (Fig. 1A) caused by scattering (measurements revealed less than 5% difference of the absolute dose calibration between the outer wells) as shown in Fig. 1B and 1C showing the radiation set up. The radiation was carried out by a 220 V unit (Gulmay D3225/GM 0196, Gulmay Medical LTD, Surrey, England) (applicator dimension 20 × 20 cm, tube current 15 mA, dose 120 MU corresponds to 1 Gy at the 4 edges of the plate, no gap between applicator and tissue culture plate). Between irradiation sessions the specimens were stored in an incubator (Binder GmbH, Tuttlingen, Germany) at 37°C in artificial saliva, which was renewed daily.

Mechanical properties evaluation/3-point flexural strength

The 3 dimensions (length, width, and height) of each specimen were measured and tested in the Universal Testing Machine (ZwickRoell) using a metallic jig inducing the load at a speed of 1 mm per minute to the center of the specimen surface until fracture. Tests were performed according to ISO 10477:1992.[16,17]

Thereafter, the flexural strength (σ in megapascals) for the rectangular sample was calculated using the following formula[17]: $\sigma = (3 \cdot Fmax \cdot L)/(2 \cdot b \cdot d^2)$, where $Fmax$ = maximal force (Newton) was applied for the fracture, L = distance (in mm) between the lower supports (span; in this study a 7-mm span was used), b = width of



1	1.000	13	1.020
2	1.044	14	1.053
3	1.049	15	1.075
4	1.049	16	1.080
5	1.049	17	1.075
6	1.013	18	1.027
7	1.020	19	0.987
8	1.062	20	1.049
9	1.071	21	1.049
10	1.084	22	1.044
11	1.066	23	1.035
12	1.027	24	0.991

C

Figure 1 (A) Radiation setting shown in 24-well acrylic cell culture plate 120MU corresponds to 1 Gy at positions 1, 2, 3, and 4 of the tissue test plate. (B) Scheme and (C) measurements of radiation dose for each well, which were filled with 1 mL natrium chloride to test dose calibration.

specimen (4 mm), and d = thickness of the specimen (1.2 mm).

Furthermore, the elasticity/flexural modulus (E in megapascals) was calculated using the following formula[17]: $E = (Fmax \cdot L^3)/(4 \cdot w \cdot t^3 \cdot y)$, where w = width of specimen (4 mm), t = thickness of the specimen (1.2 mm), and y = deflection at load point.

Scanning electron microscopy

Hemisections of the same teeth were assigned to 3 groups (A, control; B, H_3PO_4 [37%, 1 minute]; C, ethylenediaminetetraacetic acid [EDTA, 5%, 1 minute]). Specimens in group A were not further treated and stored in artificial saliva. Both hemisections (the irradiated and the nonirradiated) in group B were stored in 37% phosphoric acid (H_3PO_4) for 1 minute, while the specimens in group C were stored in 5% EDTA for 1 minute. Afterward they were rinsed with distilled water to remove the smear layer.

The preparation procedure of biological specimens for visualization under the SEM (JSM-6060, JEOL, Tokyo, Japan) included chemical fixation in glutaraldehyde followed by dehydration in ascending acetone series (50-70-80-90-96-100%) using different durations (2 × 15 minutes, 2 × 15 minutes, 2 × 15 minutes, 2 × 15 minutes, 3 × 20 minutes, 2 × 1 hour). After air drying at room temperature for 24 hours in a desiccator, they were mounted on aluminum stubs and gold/palladium sputter coated for 10 nm (90 seconds, 45mA; Balzers SCD 030, Balzers, Liechtenstein).

Scanning electron microscopy images were obtained at 10 kV, × 1000, × 5000, × 10,000, × 20,000, and × 50,000 magnification (Zeiss Supra V50, Carl Zeiss, Oberkochen, Germany).

Histologic evaluation

The specimens were dehydrated in ascending acetone series (70-80-90-96-100%), embedded in embedding resin EPON, cut with the microtome set at 3 mm and then stained in Periodic acid Schiff and toluidine blue .

The tests were performed in a Leica DM-RBEA microscope (× 1000; Leica, Wetzlar, Germany) equipped with an image system (Q-500MCA; Leica). Digital microscope images were made at increasing magnifications (× 5, × 10, × 20, and × 40).

Immunohistochemistry evaluation

Characterization of dentin tissue using rabbit COL1A2 antibodies was performed on histologic sections. The specimens were fixed in buffered formaldehyde (4%) for 1 day, demineralized in EDTA (12.5%) for 2 weeks, and embedded in paraffin. Afterward, they were sectioned and immunohistochemically stained. Therefore, the specimens were incubated with polyclonal rabbit anti-Col I antiserum (Nordic Biosite AB, Täby, Sweden) at 1/100 dilution overnight at 4°C. Specimens were counterstained with hematoxylin staining.

Statistical analysis

Statistical analyses of control and postirradiated specimens were performed by using SPSS, version 18.0 (IBM, Armonk, NY). Kolmogorov-Smirnov and Shapiro-Wilk/Weibull tests were used to test data normal distribution. A t2-way analysis of variance test revealed the statistical significance between the 2 radiation groups, and a Wilcoxon test was performed to determine significance between the control and irradiated specimens of each group. The tested variables were Fmax, flexural strength, and elasticity modulus. P values smaller than .05 were considered to be statistically significant for all comparisons.

Results

Mechanical properties analysis

Significance could be found considering the Fmax applied to break the specimens when the irradiated groups were compared with their control groups, while no significance could be found when both irradiated groups were compared with each other regarding Fmax (Tables 1 and 2). The mean Fmax values for the control groups were 108.2 MPa for group A, 72.1 MPa for group B, and 75.2 MPa for group C. Group A showed lower mean values than C, yet no significance between both

Table 1 Cross-comparison of significant differences among Δmaximal force, Δflexural strength, and Δelasticity modulus for groups A, B, C, and D based on 2-way analysis of variance test (Wilcoxon test α = 0.05)

Parameter	Group A/B	Group C/D	Group A/C	Group B/D
Fmax	**<0.0001**	**0.008**	0.173	0.088
Flexural strength	**<0.001**	0.122	**0.022**	**0.179**
Elasticity modulus	0.367	0.91	0.051	0.289

For group descriptions, see Fig. 5. Statistically significant values are presented in bold.

groups was observed. Regardless of the radiation method, Fmax decreased significantly compared with control measurements.

Flexural strength showed significant difference for the irradiated group A compared with its control group B and for the irradiated groups A and C compared with each other. Compared with its control group, irradiated group C showed no significance to D. The mean value of flexural strength for the control groups was 236.3 MPa, while for group A it was 170.2 MPa, and for group C it was 174.9 MPa. Group A showed lower mean values than C; significance was observed.

After radiation, elasticity modulus differences showed no significance, neither when irradiated groups were compared with each other nor to their control groups. The elasticity modulus values for the control groups varied between 2.7 and 13.6 MPa (mean, 7.4 MPa), while they varied between 3.6 and 12.9 MPa (mean, 5.9 MPa) for group A and between 2.5 and 12.2 MPa (mean, 7.4 MPa) for group C. Group A showed lower mean values than C, showing significant differences.

The mean and standard deviations of ΔFmax, Δflexural strength, and Δelasticity modulus values are presented in Table 1. The results of the Weibull statistics is presented in Table 2 and Fig. 2.

SEM findings

Scanning electron microscopy images indicated alteration in the tooth substance micromorphology after radiation whether with low and frequent doses or with high and less frequent doses. Irradiated specimens showed changes in observable number and distribution of dentin canals in contrast to their control specimens.

The inner structural morphology of the dentine canals was affected by the radiation. Pulpal morphology alteration could also be observed (Fig. 3).

Using pretreatment methods as EDTA and H_3PO_4 allowed the inspection of the fiber morphology by eliminating the debris resulting from the cutting procedure.

Histologic and immunohistochemical findings

Digital images were made from specimen surfaces before and after radiation and staining or immunocytochemical treatment. Control specimens showed distribution of the number and canals per area, while treated specimens presented less and uneven distributed dentin canals. The antibodies showed a netlike even binding pattern. The irradiated specimens lost the binding pattern. Immunohistochemical images can be found in Fig. 4, and SEM images are illustrated in Fig. 5.

Table 2 Mean and standard deviations of Δmaximal force (N), Δflexural strength (MPa), and Δelasticity modulus (MPa) values for the groups A and C compared with their control groups B and D

Group	Substrate	Radiation method	Produced/pretest failures/final analyzed specimens	ΔFmax (mean ± SD)	Weibull modulus (m) (95% CI)			ΔFS (mean ± SD)	Weibull modulus (m) (95% CI)			ΔEM (mean ± SD)	Weibull modulus (m) (95% CI)		
					Shape	Scale	*P*		Shape	Scale	*P*		Shape	Scale	*P*
A	Dentin	3 × 9 Gy	15/0/15	8.32 ± 28.53	4.034	126.1	>.250	90.34 ± 34.79	5.154	283.9	>.250	0.56 ± 1.65	4.474	5.692	.136
B	Dentin	None	15/0/15		5.626	110.9	>.250		5.260	230.3	.068		5.493	10.50	>.250
C	Dentin	30 × 2 Gy	15/0/15	27.84 ± 32.82	3.026	80.27	>.250	40.53 ± 82.01	3.277	188.1	>.250	2.32 ± 4.18	2.560	6.460	.018
D	Dentin	None	15/0/15		2.669	85.48	>.250		2.995	198.9	>.250		2.826	8.728	>.250

Abbreviations: ΔEM = Δelasticity modulus; ΔFmax = Δmaximal force; ΔFS = Δflexural strength; CI = confidence interval; SD = standard deviation.
Weibull modulus per experimental group analyzed after 3-bending fracture tests.

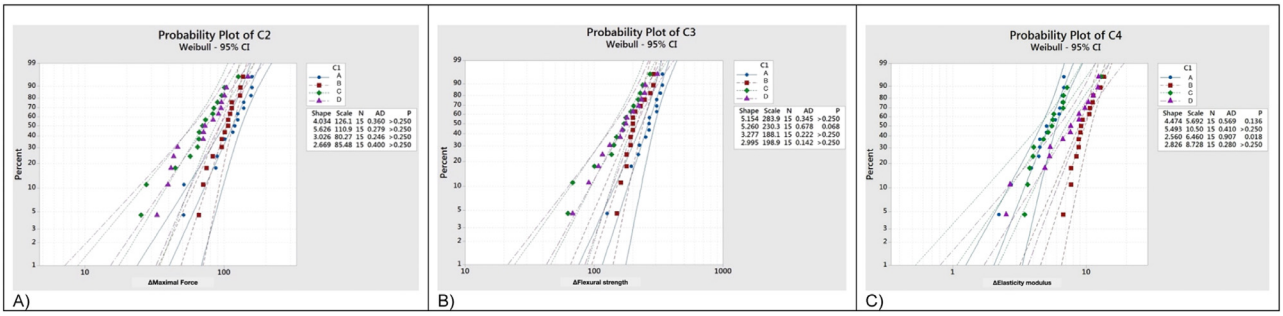


Figure 2 Weibull graph and moduli for experimental groups A, B, C, and D for the parameters (A) Δmaximal force (N), (B) Δflexural strength (MPa), and (C) Δelasticity modulus (MPa).

Discussion

The present study was conducted to investigate the effect of cumulative ionizing radiation on mechanical properties of dentin and to show structural and morphologic alterations in terms of fracture strength, flexural modulus, and elasticity modulus in dentin after variable radiation doses and frequencies based on an in vitro study in extracted third molars. Considering the obtained results, the null hypothesis was partially accepted for the flexural outcomes and rejected for the elasticity properties.

Nowadays, head and neck cancer are the sixth most prevalent cancer with an approximate incidence of 600,000 cases a year in the world.[18] Although various radiation therapy techniques have been introduced, intensity modulated radiation therapy is currently the treatment of choice because it allows precise dosing of tumoral tissue and provides greater protection of adjacent healthy structures by applying doses ranging from 30 to 70 Gy, depending on tumor type and adjuvant tissue.[19] Given this range, the applied dose for this study was 60 Gy. The ionizing radiation targets tumor cell death and operates through formation of free radicals of hydrogen and hydrogen peroxide, which can interact with water as an oxidizer and cause molecular denaturation.[19–21]

Among others, 2 of the most common oral complications of radiation therapy are hyposalivation and xerostomia, which can affect the buffering and remineralization capacity of oral tissues. The reduced salivary flow may cause a change in the oral pH. Tooth enamel becomes prone to demineralization when the oral pH drops to 5.5 or less.[22–24] The normal state of the enamel surface depends on the continuous demineralization and remineralization processes in the oral environment. When demineralization predominates, mineral losses and damages to hydroxyapatite and matrix decomposition can occur.[22,25,26] In addition to the involvement of the salivary glands, it has been suggested that radiation can cause a change in the mechanical and surface properties of teeth, especially dentin, due to the high amount of water inside

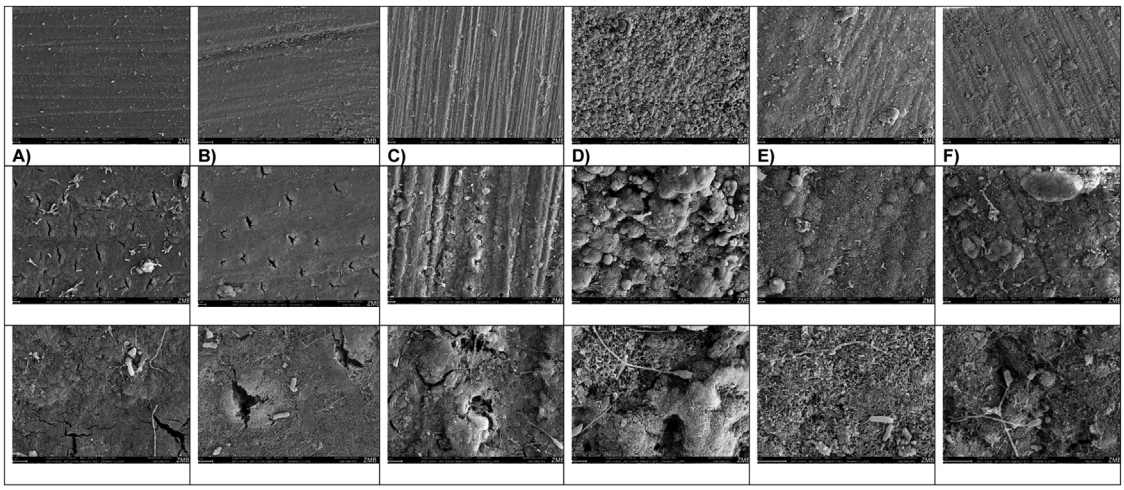


Figure 3 Scanning electron microscopy images of (A-C) outer (peripheral) and (D-F) internal (pulpal) specimen side for (A, D) control specimen, (B, E) irradiated specimen (30 × 2 Gy), and (C, F) irradiated specimen (3 × 9 Gy) at 3 different magnifications (× 1000, × 5000, × 20,000).

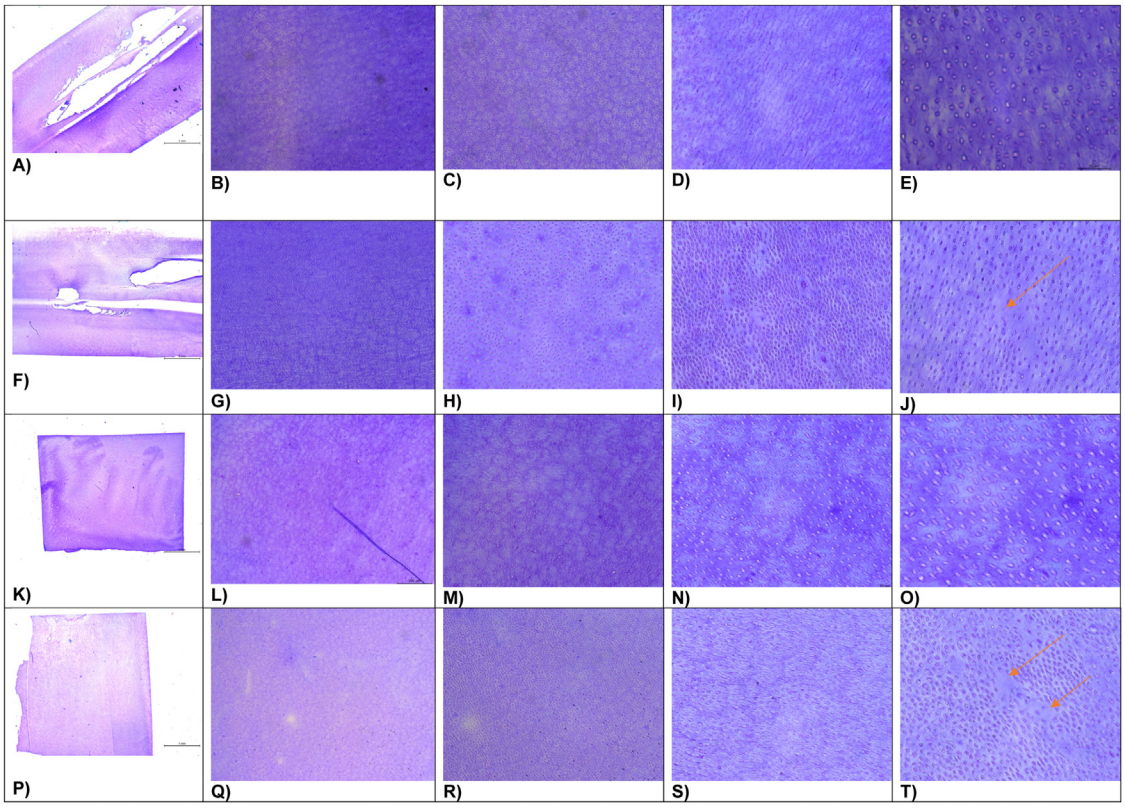


Figure 4 Digital microscope images of immunohistochemically prepared and nontreated/control specimen (A, K) at the magnifications (B, L) × 5, (C, M) × 10, (D, N) × 20, and (E, O) × 40 versus an irradiated specimen (30 × 2-Gy dose) (F) at the magnifications (G) × 5, (H) × 10, (I) × 20, and (J) × 40 versus an irradiated specimen (3 × 9-Gy dose) (P) at the magnifications (Q) × 5, (R) × 10, (S) × 20, and (T) × 40. Specimens (A-J) and (K-T) belong to the same 2 teeth. Arrow: less and uneven distributed dentin canals in irradiated specimens.

the structure, damaging the tissue by the changes in organic structures and collagen fibers.[21,27,28] This study provides robust support for this theory, demonstrating that the dental structures in irradiated patients are compromised. The irradiation affected the mechanical, biological, and physical properties of dentin. In addition, the adhesive capacities can be damaged by the biological decomposition of the collagen fibrils.[4,21,29]Considering the obtained results, the present study, confirmed and coincided with the previous literature, in which radiation was reported to cause alterations in dental tissues, directly affecting the mechanical and morphologic characteristics. The mechanical properties evaluation showed that radiation causes reduction of dental tissues and impairment of mechanical properties, such as hardness, flexural strength, and elasticity modulus.

Cumulative radiation decreases the amount of organic matrix of the enamel through the degradation of reactive oxygen species of the intertubular and intratubular structure. In addition, irradiation causes obliteration of the dentinal tubules, dehydration of collagen, and alteration of secondary and tertiary structures of the proteins. Therefore, it can be hypothesized that radiation therapy should decrease flexural strength and flexural modulus of the tooth substances dentin and enamel.

The mechanical analysis showed a statistically significant variation in the Fmax values in the group of irradiated dentin compared with those in the control group. This finding is in line with other studies in which a reduction in the microhardness of the enamel and dentin regions has been observed when subjected to the cumulative radiation doses up to 60 Gy.[4,30] Several studies confirmed that changes on the teeth produced due the radiation therapy alter the mechanical properties of these tissues.[31]

Likewise, the flexural strength showed significantly decreasing results for irradiated dentin compared with control groups. These data coincided with a previously published study by Franzel et al, who reported a decrease in the hardness of enamel and dentin along with a decrease in the elastic modulus of enamel and dentin after 60 Gy in vitro irradiation.[32] The elasticity modulus did not show any significant differences between irradiated and nonirradiated dentin but did show decreased modulus of elasticity in irradiated dentin. These data are confirmed with other studies in which there was a reduction

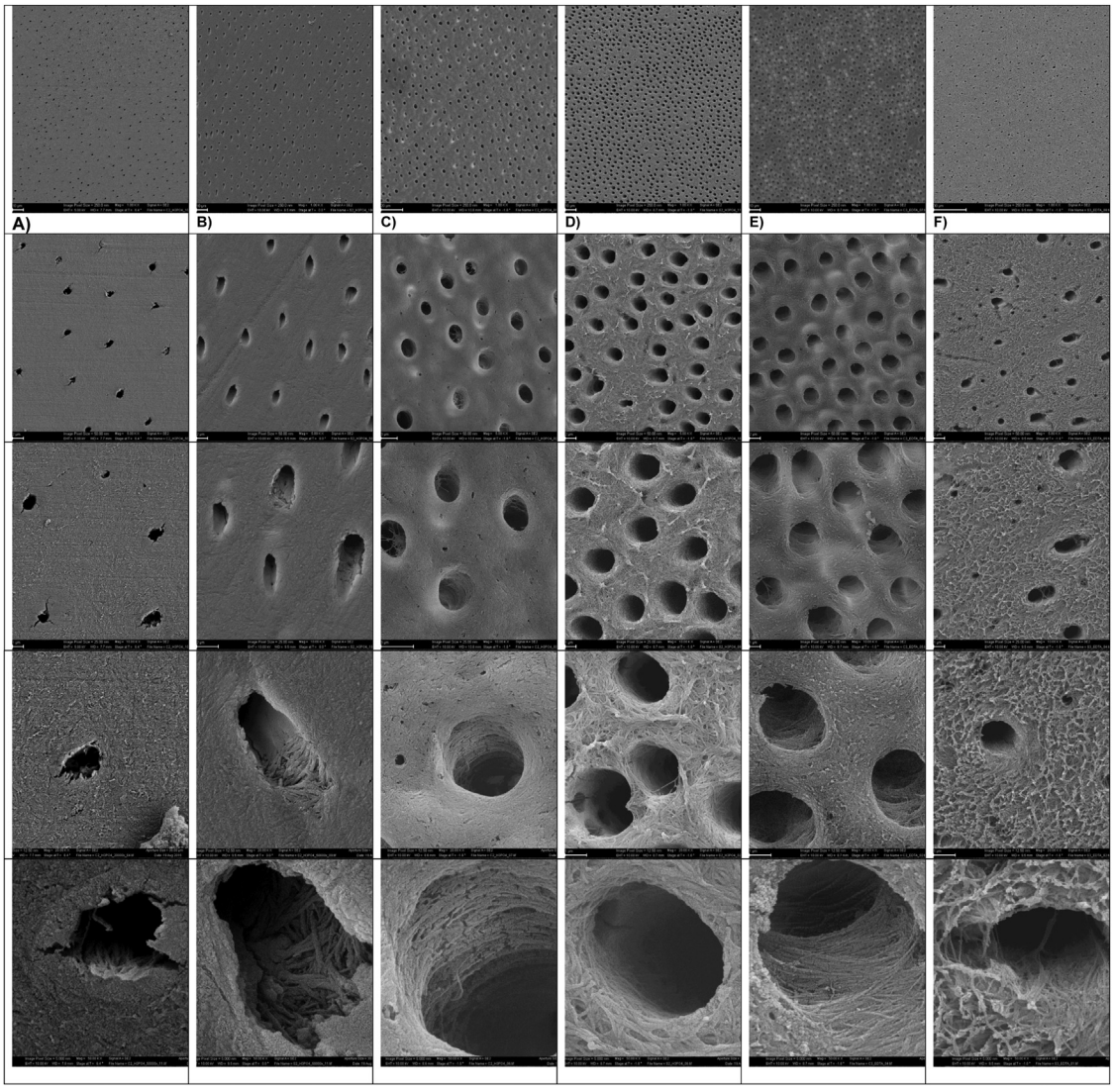


Figure 5 Scanning electron microscopy images of (A) control (nonirradiated), (B) irradiated, (C) control (nonirradiated, H_3PO_4 treated), (D) irradiated (30 × 2 Gy, H_3PO_4 treated), (E) control (nonirradiated, EDTA treated), and (F) irradiated (3 × 9 Gy, EDTA treated) specimens at 5 different magnifications (× 1000, × 5000, × 10,000, × 20,000, × 50,000).

in the elastic modulus of enamel by 60% and 45% in dentin.[32,33,]

The decrease of these mechanical properties in the enamel could be related to changes in the interaction between the organic matrix and the apatite crystals and micro cracks formation in the hydroxyapatite minerals.[31] These changes at the mechanical level are induced by the changes in their structure and composition and can lead to fractures of the teeth. In addition, radiation could affect the teeth proprioception in humans and influence biting forces, which together with the weakening of the teeth would be another risk factor for fractures.

Considering the dental surface findings using histologic and immunohistochemical analyzes, alterations of the micromorphology of dental surfaces and in the antibodies could be observed. Radiated specimens showed changes in the observable number and distribution of dentin canals in contrast to their control specimens. A massive demineralization of the teeth, especially in dentin, could be observed after radiation therapy. In some other studies, it was reported that signs of destruction of the prismatic structure and remineralization of the damaged tissue were evident.[31]

Based on the findings, there was a decrease in the organic matrix of the enamel, and the reactive oxygen species degrade the tubular structure, obliterate the dentinal tubules, dehydrate the collagen, and alter the secondary and tertiary structures of the proteins.[31] Clinically, this situation may lead to a decrease in flexural strength and the aforementioned modulus of tooth flexion.

One in vitro study, in which extracted third molars were irradiated with up to a cumulative 31.5-Gy dose during 5 days, mentioned that no measurable destruction of collagen could be detected.[34] This phenomenon was also

observed in this study, as alterations with few high doses affected the collagen structures less compared with more frequent low doses. The limit of measurable matrix transformation and, thereby, significant poorer mechanical properties is still unknown and needs further investigation. The changes in the surface of the teeth observed in the anatomy of the dentinal tubules can affect the adhesive capacity of the teeth and the future hybrid layer, which may compromise bonding strength of future restorations.

Observing the histologic and immunohistochemical results, irradiated samples presented dentin channels distributed less evenly and loss of the binding pattern of the antibodies compared with the control group. Immunohistochemistry was performed with rabbit COL1A2 antibodies binding to type I collagen, a member of collagen group I (fibril-forming collagen). Type I collagen is responsible for formation the fibrils of tendon, ligaments, and bones. In bones, the fibrils are mineralized with calcium hydroxyapatite. The C-terminal propeptide, also known as COLFI domain, has crucial roles in tissue growth and repair by controlling both the intracellular assembly of procollagen molecules and the extracellular assembly of collagen fibrils. It binds a calcium ion, which is essential for its function.[35] Considering these findings and the adhesion properties, the lack of inorganic content in the enamel could make it difficult to achieve a stronger adhesion capacity, while a higher organic content in dentin could make bonding more problematic.[36]

Accordingly, in the present study, the changes on the network-like binding pattern could negatively influence the characteristics of the dental surface because it has been shown that dentine collagen fibrils contain inactive forms of matrix metalloproteinase (MMP) proteolytic enzymes (MMP-2, -3, -8, -9 and -20) that form in the physiological and pathologic processes in dentin. Furthermore, the most important negative factor affecting the resin-dentin bond has been reported to be the incomplete infiltration of the resin into the acid-etched dentin surfaces and deterioration of the interfacial bonding of the resin-dentin interface.[37] The degradation of the resin-dentin bond caused by radiation could be complicated by the absence of the collagen fibrils necessary in the hybrid layer after the application of total or self-etch acid etch systems, causing catastrophic failures.[38–40] The use of protease (MMP) inhibitors, such as chlorhexidine is advised in case of bonding procedures of resin composite or partial and full crowns in irradiated people, as it was demonstrated that it would prevent the collagenous breakdown at the hybrid layer.[41–44]

Future in vitro studies should consider the simulation of the xerostomia experienced by patients during radiation therapy by reducing the saliva storage time and daily application of neutral sodium fluoride, which is applied in splints during radiation therapy, to reduce the side effects. However, the extent of the prevention and treatment possibilities through dental rehabilitation of irradiated humans needs further animal studies and clinical investigations with a focus on all dental hard tissues, such as enamel and dentin, and the pulp to simulate the in vivo situation, as the extracted teeth specimens do not receive a nutritional biology supply compared with the in vivo scenario.

When limitations are considered, the study indicated that radiation treatment using cumulative frequent low doses alters the anatomy of the dentin tubules by reactive oxygen species degradation of the tubular structure, obliteration the dentinal tubules, and dehydration of the collagen. A decrease of dentin flexural strength compared with single high doses is more frequent. The elasticity modulus of dentin showed no alteration after radiation treatment. The changes in the surface of the teeth observed in the anatomy of the dentinal tubules can affect the adhesive capacity of the teeth and the future hybrid layer, which may compromise bonding strength of future restorations when using resin composite, amalgam, glass ionomer cements, and resin modified glass ionomer cements as restoration materials.

Conclusion

Considering the clinical relevance in dental rehabilitation of patients with a history of radiation therapy of the oral cavity, clinicians should be aware of the increased risk of tooth fracture and retention loss of fillings and reconstructions.

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