Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth

Ligia Maria Napolitano Gonçalves [a], *Regina Guenka Palma-Dibb* [a], *Francisco Wanderley Garcia Paula-Silva* [a], *Harley Francisco de Oliveira* [b], *Paulo Nelson-Filho* [a], *Léa Assed Bezerra da Silva* [a], *Alexandra Mussolino de Queiroz* [a,*]

[a] *School of Dentistry of Ribeirao Preto at University of Sao Paulo, Brazil*
[b] *School of Medicine of Ribeirao Preto at University of Sao Paulo, Brazil*

ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form
21 May 2014
Accepted 23 May 2014

Keywords:
Radiotherapy
Permanent dentition
Microhardness
Scanning electron microscopy

ABSTRACT

Objectives: To evaluate, *in vitro*, the effects of ionizing radiation on the mechanical and micro-morphological properties of enamel and dentin of permanent teeth.
Methods: Enamel and dentin microhardness ($n = 12$ hemi-sections) was evaluated at three depths (superficial, middle and deep) prior to (control) and after every 10 Gy radiation dose up to a cumulative dose of 60 Gy by means of longitudinal microhardness. Data were analyzed using two-way analysis of variance and Tukey's test at a significance level of 5%. Enamel and dentin morphology was assessed by scanning electron microscopy (SEM) for semi-quantitative analysis ($n = 8$ hemi-sections). Data were analyzed using Kruskal–Wallis and Dunn's or Fisher exact tests at a significance level of 5%.
Results: The application of ionizing radiation did not change the overall enamel microhardness, although an increase in superficial enamel microhardness was observed. The micro-morphological analysis of enamel revealed that irradiation did not influence rod structure but interprismatic structure became more evident. Dentin microhardness decreased after 10, 20, 30, 50 and 60 Gy cumulative doses ($p < 0.05$) compared with non-irradiated dentin, mainly in the middle portion of the tissue. The micro-morphological analysis revealed fissures in the dentin structure, obliterated dentinal tubules and fragmentation of collagen fibers after 30 and 60 Gy cumulative doses.
Conclusions: Although ionizing radiation did not affect the enamel microhardness of permanent teeth as a whole, an increase in superficial enamel microhardness was observed. Dentin microhardness decreased after almost all radiation doses compared with the control, with the greatest reduction of microhardness in the middle depth region. The morphological alterations on enamel and dentin structures increased with the increase of the radiation dose, with a more evident interprismatic portion, presence of fissures and obliterated dentinal tubules, and progressive fragmentation of the collagen fibers.
Clinical significance: This study shows that irradiation affects microhardness and micro-morphology of enamel and dentin of permanent teeth. The effects of gamma irradiation on dental substrate might contribute to increased risk of radiation tooth decay associated with salivary changes, microbiota shift and high soft and carbohydrate-rich food intake.

* *Corresponding author at*: Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo, Avenida do Café, s/n, 14040-904 Ribeirão Preto, SP, Brazil. Tel.: +55 16 3602 4116; fax: +55 16 3602 4102.
E-mail address: amqueiroz@forp.usp.br (A.M. de Queiroz).
http://dx.doi.org/10.1016/j.jdent.2014.05.011
0300-5712/

1. Introduction

Approximately 500,000 head and neck new cancer cases are diagnosed every year worldwide[1] and the squamous cell carcinoma is the predominant histological type, accounting for over 90% of the cases.[2] Radiation therapy is a treatment modality that uses ionizing radiation as a therapeutic agent.[3] It is widely employed for the treatment of head and neck cancer, as an adjuvant primary therapy to surgical treatment in conjunction with chemotherapy or as a palliative treatment for advanced or inoperable tumors.[4]

The most common manifestations or complications of radiation therapy in the head and neck region are xerostomia, mucositis, candidiasis, dysgeusia, loss of taste, muscular trismus, vascular alterations, osteoradionecrosis and radiation caries. Radiation caries is a complex and destructive multifactorial disease that affects patients undergoing radiation therapy in the head and neck region.[5] This is the main complication of radiation therapy in this region, with patients presenting an increased risk for developing radiation caries for their lifetime and not only during or immediately after treatment.[6,7]

It has been reported that radiation therapy in the head and neck region has direct and indirect effects that may increase the predisposition to the development and progression of radiation caries. Indirect effects include changes in salivary flow rate and quality of saliva, difficulty in performing adequate oral hygiene, adoption of a soft diet due to difficult swallowing, and changes in oral microbiota.[7–9] Radiation therapy may also exert direct effects on the teeth, including changes in the crystalline structure, dentinoenamel junction, acid solubility of enamel, and enamel and dentin microhardness.[4–7,9–18] Unfortunately, the mechanisms leading to the development of dental caries after radiation therapy in the head and neck region remains unclear.[6,16]

The aim of this study was to evaluate *in vitro* the effects of ionizing radiation on the mechanical and micro-morphological properties of enamel and dentin of permanent teeth by the analysis of longitudinal microhardness and scanning electron microscopy (SEM).

2. Materials and methods

This study was submitted to the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, and initiated after being approved. The extracted sound human molars that were used in this study come from the Dental Bank of the School of Dentistry of Ribeirão Preto, University of São Paulo.

Twenty healthy freshly extracted human first permanent maxillary ($n = 10$) and mandibular ($n = 10$) molars were obtained and stored in distilled water at 4 °C for a period of no longer than 1 month. The roots were removed approximately 5 mm below the cementoenamel junction and the teeth were bisected longitudinally in a mesiodistal direction. The buccal halves ($n = 20$ hemi-sections) were used for analysis of microhardness ($n = 12$ hemi-sections) and surface micromorphology ($n = 8$ hemi-sections) of enamel and dentin before (control) and after ionizing radiation. As a standard laboratory protocol, the fragments were cleaned and their pulpal side was ground wet with 600- and 1200-grit silicon carbide papers in a polishing machine (DP-9U2; Panambra/Strues A/S, Copenhagen, Denmark) and polished with felt discs (Diamond, FGM, Joinvile, SC, Brazil) embedded in aluminium oxide paste (Alpha micropolish LC, Union Carbide Corp., Houston, TX, USA). The specimens were washed in running water, dried with gauze and examined at ×40 magnification to confirm their smoothness. Then, the specimens were placed in ultrasonic in water for 5 min to remove possible debris. Measurement of microhardness is only possible on smooth enamel and dentin surfaces because the indentations are not visible on non-polished surfaces. The polished hemi-sections were placed in a 24-well acrylic cell culture plate, and each well was filled with 10 mL of artificial saliva in a way that all specimens could receive the same direct ionizing radiation *per* unit area.

Initial microhardness of enamel and dentin was evaluated before irradiation of the specimens. The test was performed with a pyramidal diamond indenter (Shimadzu Micro Hardness Tester HMV-2000-Corporation, Kyoto, Japan) to produce an elongated diamond-shaped indent for Knoop hardness (KH) under 10 gf load and 15 s dwell time in dentin and 25 gf load and 10 s dwell time in enamel. Indentations were made in 3 regions of enamel: the first at 50 μm from its outer border (surface enamel), the second at one-half the thickness of enamel (middle enamel), and the third at 50 μm from the dentinoenamel junction (deep enamel). The dentin indentations were made at 50 μm from the dentinoenamel junction (surface dentin), at one-half the thickness of dentin (middle dentin), and at 50 μm from the pulp chamber (deep dentin). In each selected region of each specimen, three indentations spaced 100 μm from each other in enamel and 150 μm in dentin were made by the same calibrated examiner, who were trained to visualize where the previous indentation was performed. The representative dentin and enamel microhardness values for each specimen were obtained as the average of the results for the three indentations.

After analysis of initial microhardness, the dental fragments were irradiated in a Cobalt unit with 1.25 MV photons (Gammatron 580, Siemens, Munich, Germany), at dose rate of 1 Gy/min, and a source-surface distance of 80 cm. A dose of 2 Gy/fraction (1 fraction per day, 5 times a week) was used, up to a cumulative dose of 60 Gy (30 fractions on a 6-weeks course). Between the radiation cycles, the fragments were stored in artificial saliva in an incubator at 37 °C, which was renewed daily. The measurements of post-irradiation enamel and dentin microhardness were conducted after every 10 Gy of radiation until completing 30 irradiation cycles (30 days), which is equivalent to a cumulative dose of 60 Gy. Microhardness measurements for each period were performed 100 μm (enamel)/150 μm (dentin) of the last measurement which was made, in spite of ionizing radiation was possible to view the previous measurements.

Control and post-irradiation tissue microhardness values were analyzed using a two-way analysis of variance and Tukey's test at a level of significance of 5%.

SEM analysis was performed using buccal hemi-sections from non irradiated (control; $n = 4$) and irradiated teeth after a cumulative dose of 30 Gy ($n = 2$) and 60 Gy ($n = 2$). The SEM prepared specimens were fixed in glutaraldehyde solution in

cacodylate buffer, cleaned for 10 minutes in an ultrasonic bath (UltrasonicCleaner T-1449-D. Odontobrás Ind. and Com, Ribeirão Preto, SP, Brazil) with distilled and deionized water, dehydrated in a series of increasing ethanol concentrations (25%, 50%, 75%, 95%, and 100%), and immersed in hexamethyldisilazane (HMDS) for 10 min. Subsequently, the specimens were fixed on stubs with a double-sided adhesive carbon tape (Electron Microscopy Sciences, Washington, PA, USA) and were sputter-coated with gold in a vacuum metallizing machine (SDC 050;Bal-Tec AG, Balzers, Germany) and examined with a scanning electron microscope (Philips XL30 FEG, Eindhoven, The Netherlands).

Control and post-irradiation tissue micro-morphological changes were analyzed according using a score system. Enamel prismatic structure was scored as follows: (0) Regular rod-like structure, (1) Slight change in rod structure, (2) Moderate change in rod structure and (3) Severe change in rod structure. Interprismatic structures changes were scored as (0) Unaltered, (1) Slight alteration, (2) Moderate alteration, (3) Severe alteration. For dentin tubules scores were attributed as follows: (0) Regular, (1) Partially obliterated, (2) Totally obliterated. Dentin collagen fiber network was scored as (0) Regular, (1) Slight alteration, (2) Moderate alteration,.(3) Presence of fissures was classified as (0) Absent or (1) Present. Score data were analyzed using Kruskal–Wallis followed by Dunn's test at a level of significance of 5%. Dichotomic data (Absence/Presence) were analyzed using Fisher exact test at a level of significance of 5%. Statistical analyses were performed as recommended elsewhere.[19]

3. Results

3.1. *Enamel and dentin microhardness*

Overall, enamel microhardness values decreased after cumulative radiation doses of 10, 20 and 30 Gy doses when compared with non-irradiated enamel (control) ($p < 0.05$). Doses higher than that did not influence the enamel microhardness ($p > 0.05$) (Table 1). In the analysis of the interacting factors, microhardness at different depths and different irradiation doses, it was observed that enamel microhardness values decreased in superficial depth up to 30 Gy cumulative dose ($p < 0.05$) but increased with doses higher than that ($p < 0.05$). In the middle enamel, microhardness did not differ significantly compared with the non-irradiated enamel after cumulative radiation doses of 10, 30, 40, 50 and 60 Gy ($p > 0.05$). In deep enamel, there was no change in microhardness ($p > 0.05$) (Table 2).

As a whole, dentin microhardness decreased after 10, 20, 30, 50 and 60 Gy cumulative radiation doses ($p < 0.05$) compared with non-irradiated dentin (control) (Table 3). In the analysis of the interacting factors, microhardness at different depths and different irradiation doses, it was observed that for the superficial dentin, microhardness had no alteration after the 10, 20, 30, 40, 50 and 60 Gy cumulative radiation doses, compared with the control ($p > 0.05$). In the middle dentin, there was a decrease in the microhardness values after the different radiation doses ($p < 0.05$). Radiation did not influence microhardness of deep dentin ($p > 0.05$) (Table 4).

Table 1 – Longitudinal Knoop microhardness mean values and standard deviations before radiation therapy (control) and after the different ionizing radiation doses, in the enamel of permanent teeth.

Control	202.43 ± 44.12^{a}
10 Gy	177.94 ± 36.01^{b}
20 Gy	165.10 ± 33.41^{b}
30 Gy	185.01 ± 29.05^{b}
40 Gy	206.40 ± 36.56^{a}
50 Gy	208.47 ± 30.42^{a}
60 Gy	207.90 ± 28.86^{a}

Different letters indicate statistically significant difference.

Table 2 – Longitudinal Knoop microhardness mean values and standard deviations before radiation therapy (control) and after the different ionizing radiation doses, at different depths of the enamel of permanent teeth.

	Superficial enamel	Middle enamel	Deep enamel
Control	204.19 ± 53.48^{b}	207.42 ± 33.03^{A}	$195.69 \pm 46.50^{▲}$
10 Gy	175.92 ± 31.70^{cd}	183.64 ± 33.14^{A}	$174.28 \pm 44.37^{▲}$
20 Gy	152.34 ± 26.31^{d}	170.42 ± 35.24^{B}	$172.54 \pm 36.73^{▲}$
30 Gy	183.37 ± 36.37^{bc}	184.89 ± 25.75^{A}	$186.78 \pm 26.33^{▲}$
40 Gy	232.05 ± 43.20^{a}	199.58 ± 21.45^{A}	$187.56 \pm 27.75^{▲}$
50 Gy	227.64 ± 32.86^{a}	207.53 ± 15.54^{A}	$190.25 \pm 29.30^{▲}$
60 Gy	230.42 ± 29.44^{a}	199.50 ± 16.66^{A}	$193.78 \pm 25.68^{▲}$

Different letters and symbols indicate statistically significant difference.

Table 3 – Longitudinal Knoop microhardness mean values and standard deviations before radiation therapy (control) and after the different ionizing radiation doses, in the dentin of permanent teeth.

Control	28.46 ± 8.19^{a}
10 Gy	23.65 ± 7.86^{c}
20 Gy	23.53 ± 6.91^{c}
30 Gy	24.97 ± 6.07^{bc}
40 Gy	26.47 ± 11.67^{ab}
50 Gy	23.72 ± 6.36^{c}
60 Gy	25.34 ± 6.34^{bc}

Different letters indicate statistically significant difference.

Table 4 – Longitudinal Knoop microhardness mean values and standard deviations before radiation therapy (control) and after the different ionizing radiation doses, at different depths of the dentin of permanent teeth.

	Superficial dentin	Middle dentin	Deep dentin
Control	26.85 ± 4.22^{ab}	36.40 ± 6.98^{A}	$22.12 \pm 5.60^{●}$
10 Gy	22.76 ± 6.23^{b}	28.39 ± 7.29^{B}	$19.81 \pm 7.94^{●}$
20 Gy	22.94 ± 6.16^{b}	28.29 ± 5.27^{B}	$19.37 \pm 6.53^{●}$
30 Gy	24.88 ± 1.94^{b}	29.19 ± 4.13^{B}	$20.84 \pm 7.65^{●}$
40 Gy	30.29 ± 17.31^{a}	28.00 ± 5.51^{B}	$21.13 \pm 7.31^{●}$
50 Gy	23.56 ± 64.73^{b}	27.97 ± 4.66^{B}	$19.62 \pm 6.83^{●}$
60 Gy	24.78 ± 3.08^{b}	29.79 ± 5.09^{B}	$21.46 \pm 7.36^{●}$

Different letters and symbols indicate statistically significant difference.

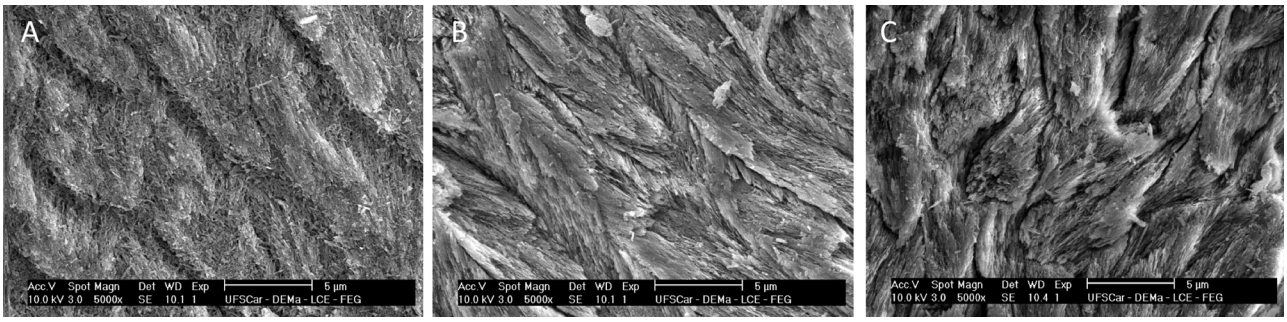

Fig. 1 – SEM micrographs of the enamel of permanent teeth (5000×). (A) Non-irradiated enamel with well organized prisms surrounded by the interprismatic regions; (B) (30 Gy) and (C) (60 Gy). Interprismatic region of irradiated enamel, showing clearly the prisms and crystals.

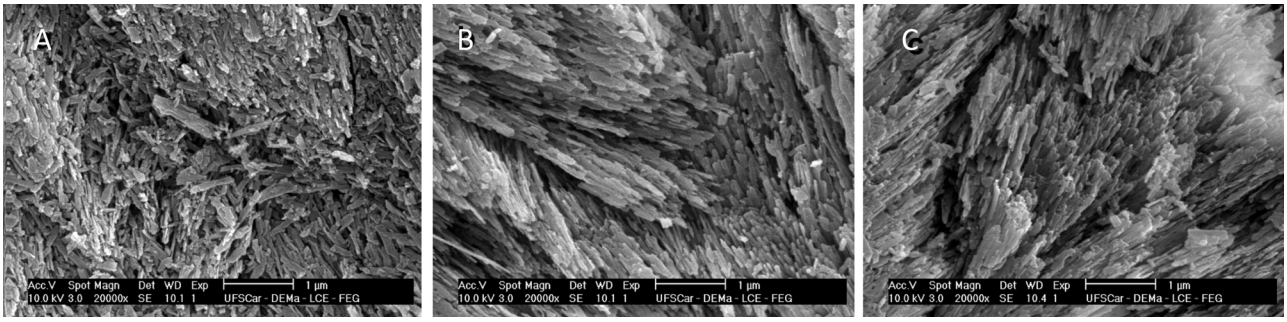

Fig. 2 – SEM micrographs of enamel of permanent teeth (20,000×). (A) Non-irradiated enamel with well organized prisms surrounded by the interprismatic regions; (B) (30 Gy) and (C) (60 Gy). Interprismatic region of irradiated enamel, showing clearly the prisms and crystals.

3.2. *SEM analysis of enamel and dentin*

The enamel of non-irradiated teeth presented well-organized prisms with transverse and oblique arrangement and surrounded by interprismatic portions (Figs. 1A and 2A). Prismatic structure of irradiated enamel remained unaltered even after application of the different radiation doses ($p > 0.05$) (Figs. 1B,C and 2B,C). A slight micro-morphological alteration was observed in the interprismatic region after 30 Gy radiation dose (Figs. 1B and 2B), which became more evident increasing the irradiation dose (60 Gy; Fig. 1C and 2C) ($p < 0.05$).

The dentin of non-irradiated teeth presented well-defined dentinal tubules and a well-organized collagen fiber network (Figs. 3A, 4A and 5A). There was an increase in the morphological alterations after 30 and 60 Gy radiation doses at all analyzed regions compared with the non-irradiated dentin ($p < 0.05$). Alterations in the intertubular, peritubular and intratubular dentin could be observed as the radiation doses increased. Starting with the 30 Gy, fissures in dentinal structure became evident at 10,000× and 20,000× magnifications ($p < 0.05$) (Figs. 3B and 4B,C). With the 60 Gy cumulative radiation dose, the dentinal tubules became obliterated

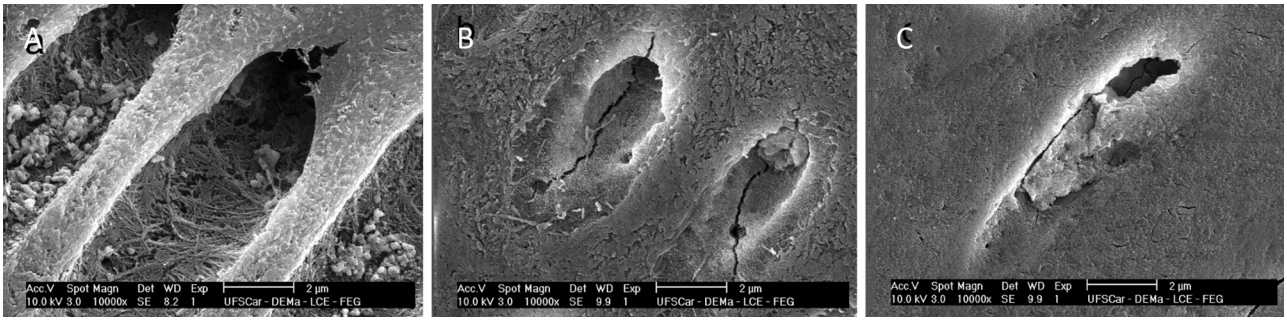

Fig. 3 – SEM micrographs of dentin of permanent teeth (10,000×). (A) Non-irradiated dentin with well defined dentinal tubules and an organized collagen fiber network; (B) (30 Gy) and (C) (60 Gy). Irradiated dentin showing alteration of the intertubular, peritubular and intratubular dentin, presence of cracks in the dentinal structure and obliterated dentinal tubules.

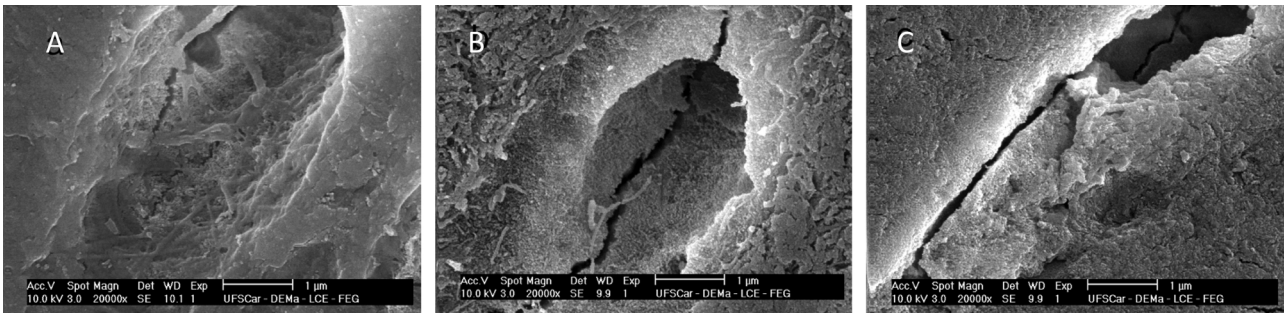

Fig. 4 – SEM micrographs of dentin of permanent teeth (20,000×). (A) Non-irradiated dentin with well defined dentinal tubules and organized collagen fibers; (B) (30 Gy) and (C) (60 Gy). Irradiated dentin showing alteration of the intertubular, peritubular and intratubular dentin, presence of cracks in the dentinal structure, obliterated dentinal tubules, and increasing destruction of collagen fibers.

Fig. 5 – SEM micrographs of dentin of permanent teeth (20,000x). (A) Non-irradiated dentin with well defined dentinal tubules and an organized collagen fiber network; (B) (30 Gy) and (C) (60 Gy). Irradiated dentin showing alteration of the intertubular, peritubular and intratubular dentin, presence of cracks in the dentinal structure, obliterated dentinal tubules, and increasing destruction of the collagen network with fragmented fibers.

($p < 0.05$) (Figs. 3C and 4C). The collagen fibers were gradually fragmented with the increase of radiation doses ($p < 0.05$). In the 20,000× magnification the fragmenetation of a collagen fibers became more evident (Fig. 5B and C).

4. Discussion

The application of ionizing radiation reduced the overall enamel microhardness up to 30 Gy, but after 60 Gy no change was observed, in agreement with the results of other studies that did not find differences in enamel microhardness after ionizing radiation.[13–15] Interestingly, considering the different radiation doses and enamel depths, superficial enamel after a 40 Gy cumulative radiation dose presented higher microhardness than non-irradiated enamel. We speculate that changes in Hunter-Schreger bands patterns[20] might occur following irradiation. Those changes might be accompanied by higher microhardness of superficial layer, which turns the enamel more friable and susceptible to crack formation, contributing to dentinal hypersensitivity and favouring marginal infiltration of restorations.

After cumulative radiation of 30 and 60 Gy, no morphological alteration in the prismatic enamel structure was observed, although the interprismatic portion became more evident with the increase of the radiation dose, as reported previously.[12,16]

It has been reported that radiation does not present direct effects on the inorganic structure of human teeth, and the observed dental alterations in patients with head and neck cancer after radiation therapy are due instead to the alterations in the organic matrix of enamel.[14,21] Indeed, SEM analysis revealed more significant morphological alterations in the interprismatic region, which corresponds to enamel organic matrix. It is likely that the alterations in the interprismatic region, which concentrates water, result from free radicals and reactive oxygen species accumulation that may react with and damage organic components. It is our understanding that alterations in the enamel organic matrix after ionizing radiation may contribute to dental problems arising after the head and neck radiation therapy.

Dentin behaved quite differently from enamel, with a decrease in dentin microhardness as a function of the radiation doses. Previously it was reported that dentin microhardness reduced following irradiation in permanent,[13] deciduous[22] or bovine teeth.[15] Here, we demonstrated for the first time that the middle region accounts for reduction of dentin microhardness in permanent teeth. These findings are

different from deciduous teeth, where the main reduction in microhardness occurs in the superficial dentin. Although the reason cannot be ascertained, we speculated that differences in dentin thickness and composition might influence microhardness.

SEM analysis of permanent tooth dentin after cumulative radiation doses of 30 and 60 Gy revealed degradation of the collagen fibers network and generalized micro-morphological alterations. In the present study, there were obliteration and fissures in the dentinal structure and fragmentation of the collagen fiber network, possibly resulting from the loss of collagen fiber hydration, leaving the tissue dry and friable. Irradiation of proteins causes alterations in their secondary and tertiary structures, with harmful effects on the hydration of collagen fibers by the action of free radicals.[23]

Ionizing radiation had different effects on the microhardness of enamel and dentin. A possible explanation could be the fact that dentin has higher water content than enamel –10% *versus* 4% by weight.[24] A known fact is that radiation acts on water, leading to formation of free radicals and hydrogen peroxide.[25] In this way, tissue with higher water content could be more vulnerable to the radiation effects than another one with lower water content and having a stronger effect on tissue's mechanical properties. This fact occurred in the present study, as generally the dentin microhardness values diminished after radiation. Micro-morphological changes in dentin could also explain the progressive decrease of microhardness with the increase of radiation dose. As dentin supports enamel, a softer dentin tissue becomes less efficient, allowing the occurrence of fractures and cracks in the enamel.

The apparent degradation of the organic portion of dentin could also interfere with the adhesion of resinous restorative materials. Further studies are required to evaluate whether the micro-morphological alterations observed in this study could influence bond strength of resin-based composite restoration reported previously.[26]

A recent review hypothesized that dental caries in irradiated teeth might be due to a combination of poor oral hygiene, increase of soft and carbohydrate-rich food intake, salivary changes, as well as direct effects on hard dental tissue.[27] Our results demonstrate that irradiation affects the micro-morphological structures of enamel and dentin accompanied by reduced dentin microhardness. Together, those changes might increased the risk of radiation caries and influence the outcome of dental treatment performed in patients with head and neck cancer submitted to radiation therapy. Research should be performed to investigate *in vivo* the effects of radiation in teeth of patients undergoing radiotherapy.

5. Conclusion

Ionizing radiation increased superficial enamel microhardness of permanent teeth. Dentin microhardness, on the other hand, reduced mainly in the middle dentin. The increase of the cumulative radiation doses resulted in progressive micro-morphological alterations of enamel and dentin structures. In enamel, the interprismatic portion became more evident, while fissures, dentinal tubules obliteration and fragmentation of collagen fibers were observed in dentin.

REFERENCES

1. Shibuya K, Mathers CD, Boschi-Pinto C, Lopez AD, Murray CJ. Global and regional estimates of cancer mortality and incidence by site: II. Results for the global burden of disease 2000. *BMC Cancer* 2002;**2**:37.
2. Casiglia J, Woo SB. A comprehensive review of oral cancer. *General Dentistry* 2001;**49**:72–82.
3. Chaachouay H, Ohneseit P, Toulany M, Kehlbach R, Multhoff G, Rodemann HP. Autophagy contributes to resistance of tumor cells to ionizing radiation. *Radiotherapy and Oncology* 2011;**99**:287–92.
4. Soares CJ, Neiva NA, Soares PB, Dechichi P, Novais VR, Naves LZ, *et al.* Effects of chlorhexidine and fluoride on irradiated enamel and dentin. *Journal of Dental Research* 2011;**90**:659–64.
5. Silva AR, Alves FA, Antunes A, Goes MF, Lopes MA. Patterns of demineralization and dentin reactions in radiation-related caries. *Caries Research* 2009;**43**:43–9.
6. Al-Nawas B, Grötz KA, Rose E, Duschner H, Kann P, Wagner W. Using ultrasound transmission velocity to analyze the mechanical properties of teeth after in vitro, in situ, and in vivo irradiation. *Clinical Oral Investigations* 2000;**4**:168–72.
7. Kielbassa AM, Hinkelbein W, Hellwig E, Meyer-Lückel H. Radiation-related damage to dentition. *Lancet Oncology* 2006;**7**:326–35.
8. Vissink A, Jansma J, Spijkervet FK, Burlage FR, Coppes RP. Oral sequelae of head and neck radiotherapy. *Critical Reviews in Oral Biology and Medicine* 2003;**14**:199–212.
9. Silva AR, Alves FA, Berger SB, Giannini M, Goes MF, Lopes MA. Radiation-related caries and early restoration failure in head and neck cancer patients. A polarized light microscopy and scanning electron microscopy study. *Supportive Care in Cancer* 2010;**18**:83–7.
10. Jansma J, Borggreven JM, Driessens FC, 's-Gravenmade EJ. Effect of X-ray irradiation on the permeability of bovine dental enamel. *Caries Research* 1990;**24**:164–8.
11. Kielbassa AM, Schendera A, Schulte-Mönting J. Microradiographic and microscopic studies on in situ induced initial caries in irradiated and nonirradiated dental enamel. *Caries Research* 2000;**34**:41–7.
12. Jervoe P. X-ray diffraction investigation on the effect of experimental and in situ radiation on mature human teeth. A preliminary report. *Acta Odontologica Scandinavica* 1970;**28**:623–31.
13. Markitziu A, Gedalia I, Rajstein J, Grajover R, Yarshanski O, Weshler Z. In vitro irradiation effects on hardness and solubility of human enamel and dentin pretreated with fluoride. *Clinical Preventive Dentistry* 1986;**8**:4–7.
14. Jansma J, Buskes JA, Vissink A, Mehta DM, Gravenmade EJ. The effect of X-ray irradiation on the demineralization of bovine dental enamel. A constant composition study. *Caries Research* 1988;**22**:199–203.
15. Kielbassa AM, Beetz I, Schendera A, Hellwig E. Irradiation effects on microhardness of fluoridated and non-fluoridated bovine dentin. *European Journal of Oral Sciences* 1997;**105**:444–7.
16. Grötz KA, Duschner H, Kutzner J, Thelen M, Wagner W. Histotomography studies of direct radiogenic dental enamel changes. *Mund- Kiefer- und Gesichtschirurgie* 1998;**2**:85–90.
17. Açil Y, Springer IN, Niehoff P, Gassling V, Warnke PH, Açmaz S, *et al.* Proof of direct radiogenic destruction of collagen in vitro. *Strahlentherapie und Onkologie* 2007;**183**:374–9.
18. Soares CJ, Castro CG, Neiva NA, Soares PV, Santos-Filho PC, Naves LZ, *et al.* Effect of gamma irradiation on ultimate tensile strength of enamel and dentin. *Journal of Dental Research* 2010;**89**:159–64.

19. Hannigan A, Lynch CD. Statistical methodology in oral and dental research: pitfalls and recommendations. *Journal of Dentistry* 2013;**41**:385–92.
20. Lynch CD, O'Sullivan VR, Dockery P, McGillycuddy CT, Sloan AJ. Hunter-Schreger band patterns in human tooth enamel. *Journal of Anatomy* 2010;**217**:106–15.
21. Zach GA. X-ray diffraction and calcium-phosphorous analysis of irradiated human teeth. *Journal of Dental Research* 1976;**55**:907–9.
22. Mellara TS, Palma-Dibb RG, Oliveira HF, Paula-Silva FWG, Nelson-Filho F, Silva RAB, *et al.* The effect of radiation therapy on the mechanical and morphological properties of the enamel and dentin of deciduous teeth – an in vitro study. *Radiation Oncology* 2014;**9**:30.
23. Chistiakov DA, Voronova NV, Chistiakov PA. Genetic variations in DNA repair genes, radiosensitivity to cancer and susceptibility to acute tissue reactions in radiotherapy-treated cancer patients. *Acta Oncologica* 2008;**47**:809–24.
24. Mjör IA. Dentin permeability: the basis for understanding pulp reactions and adhesive technology. *Brazilian Dental Journal* 2009;**20**:3–16.
25. Cole T, Silver AS:. Production of hydrogen atoms in teeth by X-irradiation. *Nature* 1963;**200**:700–1.
26. Naves LZ, Novais VR, Armstrong SR, Correr-Sobrinho L, Soares CJ. Effect of gamma radiation on bonding to human enamel and dentin. *Supportive Care in Cancer* 2012;**20**:2873–8.
27. Lieshout HF, Bots CP. The effect of radiotherapy on dental hard tissue – a systematic review. *Clinical Oral Investigations* 2014;**18**:17–24.