Original Research
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How to improve root canal filling in teeth subjected to radiation therapy for cancer

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Abstract: The aim of this study was to evaluate the influence of radiation therapy on root canal sealer push-out bond strength (BS) to dentin and the sealer/dentin interface after different final irrigation solutions (NaOCl, EDTA, and chitosan). Sixty-four maxillary canines were distributed into two groups (n=30): non-irradiated and irradiated with 60 Gy. Canals were prepared with Reciproc-R50 and subdivided (n=10) for final irrigation (NaOCl, EDTA, chitosan) and filled. Three dentin slices were obtained from each root third. The first slice of each third was selected for BS evaluation, and the failure mode was determined by stereomicroscopy. SEM analysis of the sealer-dentin interface was performed in the remaining slices. Two-way ANOVA and Tukey's tests (α=0.05) were used. Lower BS ($P<0.0001$) was obtained after irradiation (2.07±0.79 MPa), regardless of the final irrigation solution used. The NaOCl group ($P<0.001$) had the lowest BS in the irradiated (1.68±0.72) and non-irradiated (2.39±0.89) groups, whereas the EDTA (irradiated: 2.14±0.77 and non-irradiated: 3.92±1.54) and chitosan (irradiated: 2.37±0.73 and non-irradiated: 3.51±1.47) groups demonstrated a higher BS ($P<0.05$). The highest values were observed in the coronal third (3.17±1.38) when compared to the middle (2.74±1.36) and apical ones (2.09±0.97)($P<0.0001$). There were more cohesive failures and more gaps in irradiated specimens, regardless of the final solution. The present study showed that radiation was associated with a decrease in BS, regardless of the final solution used, whereas chitosan increased BS in teeth subjected to radiation therapy.

Keywords: Radiotherapy; Dental Pulp Cavity; Chitosan.

Introduction

Head and neck cancer ranks seventh among the most common neoplasms worldwide, with an annual incidence of approximately 640,000 new cases.[1,2,3] When diagnosed early, oral cancer presents a survival rate between 80% and 90%;[4] however, most neoplasms are diagnosed late and the survival rate drops to 57% within the first 5 years after diagnosis.[4]

Treatment for head and neck cancer depends on factors such as the type of cancer, staging, and location,[5] and can be accomplished by surgery, radiation therapy, chemotherapy, or a combination thereof.[5] Radiation therapy acts directly on the DNA by inhibiting cell division, or indirectly, by producing free radicals and resulting in cellular necrosis.[5] Conventional radiation fractionation is the most commonly regimen used to minimize the side effects of radiation therapy on healthy tissues, favoring their repair.[6,7] However, adjacent tissues are rarely preserved during head and neck radiation therapy,[8] and this may vary according to the patient's age and to the dose and location of the ionizing radiation.[5,9]

Some complications can be acute, such as pain and soft tissue sensitivity, qualitative and quantitative changes in saliva, loss of taste, fungal infections, and mucositis,[10] whereas others develop later, such as xerostomia, mandibular osteoradionecrosis, muscular atrophy, trismus, radiation caries, changes in the bacterial microflora, and ultrastructural alterations in dentin.[9,10,11]

Structural changes in enamel and dentin[12] and direct damages to collagen,[13,14] as well as reduction of dentin microhardness,[14] favor the development and progression of radiation caries, which may lead to pulpal changes[9,11,15] and result in the need for endodontic treatment.[11]

According to Martins et al.,[11] radiation therapy performed before endodontic treatment reduces bond strength of the filling material to root dentin, regardless of the sealer type used, once it damages the dentin collagen fiber network.[11,13,14]

Recently, chitosan has been proposed as a final irrigation solution for the treatment of dentin surface and removal of the smear layer,[16,17]with the increase in collagen fiber degradation by collagenase,[18] which may improve the long-term stability of exposed fibers inside the root canals during endodontic treatment.[18] Therefore, considering radiation-induced changes in the collagen fiber network in the intertubular, peritubular, and intratubular dentin[11] and the ability of chitosan to stabilize the collagen structure, it is important to evaluate the behavior of different final irrigation solutions before root canal filling of teeth undergoing radiation therapy.

The aim of this study was to assess the push-out bond strength of epoxy resin-based sealer to intraradicular dentin after final irrigation with different solutions (NaOCl, EDTA, and chitosan), as well as the sealer/dentin interface in teeth subjected to fractionated radiation.

Methodology

Sample selection

After approval by the Research Ethics Committee of the Dental School of Ribeirão Preto, University of São Paulo, Brazil, protocol no. 53666016.0.0000.5419, 60 straight, single-rooted human maxillary canines with complete rhizogenesis, absence of calcifications and internal resorption, no previous endodontic treatment, as well as absence of metallic restorations,[11] with root length of at least 17 mm were selected from the endodontic laboratory collection. The teeth were kept in individual and labeled plastic vials with artificial saliva (pH 7.0, 37°C) and renewed daily.

Irradiation protocol

The teeth were randomly distributed into two groups (n = 30): one group without irradiation, and the other one was subjected to fractionated radiation with 6 MV X-rays. The teeth prepared to receive radiation were kept inside plastic vials with distilled water to obtain a uniform radiation dose (approximately 2.85 Gy/min).[11,14] Treatment was done using a computer-assisted linear accelerator with 6 MV X-rays (RS 2000, RAD Source Technologies, Inc., Suwanee, USA) with energy of 200 kVp and 25 mA and a 0.3-mm standard copper filter. The cumulative radiation dose of 60 Gy was divided into 30 fractions (2 Gy/fraction) and delivered in 5 consecutive days/week, for 6 weeks. Between the irradiation cycles, the samples were stored in artificial saliva as previously described.

Root canal preparation and filling

Conventional endodontic cavities were made and the root canal was irrigated with 1% NaOCl (2 mL). The coronal thirds were serially prepared up to a 45.06 taper size with an LA Axxess bur (SybronEndo Corporation, Orange, USA). The working length was determined at 1.0 mm of the apical foramen.

Reciproc R50 (taper size 50.05) files were used in a reciprocating motion by the VDW Silver motor (VDW GmbH, Munich, Germany). The root canals were prepared using an in-and-out pecking motion (3 mm in amplitude and light apical pressure) and cleaned after three pecking motions, following the manufacturer's recommendations. The irrigation was performed with 2 mL of 1% NaOCl using a plastic syringe and a 30 G needle during each step.

After biomechanical preparation, the teeth were redistributed into three subgroups (n=10) according to the final irrigation solution used (5 mL): Subgroup A - 1% NaOCl (control), Subgroup B - 17% EDTA, and Subgroup C – 0.2% chitosan, for 5 min. Root canals were then flushed using 2 mL of distilled water and R50 absorbent paper cones (Dentsply Maillefer, Baillagues, Switzerland) were used for drying. After that, the canals were filled using the lateral condensation technique (R50 and R8 accessory cones) with AH Plus sealer (Dentsply, De Trey, Konstanz, Germany). Endodontic access was temporarily restored with a self-setting filling material (Coltosol, Coltène\Whaledent, S.A., Alstatten Switzerland) and kept at 95% humidity and 37 °C for a period of three times longer than the AH Plus sealer setting time (8 h).

Bond strength and SEM analysis

The roots were sectioned perpendicularly into 1-mm-thick slices with a water-cooled low-speed diamond disc (Isomet 1000; Buehler, Lake Forest, USA), obtaining three slices from each root third.

The first slice was used for the push-out test in a universal testing machine (Instron 2519-106; Instron, Canton, USA) at a crosshead speed of 0.5 mm/min^{-1}. The apical surface was placed towards the tip, making sure forces were applied from the apical to the coronal direction. Four-millimeter-long shafts with tip diameters of 0.4 mm, 0.6 mm, and 1.0 mm were used, respectively, for the apical, middle, and coronal root slices. A constant load was applied until displacement of the root filling material. The bond strength was determined in MPa by dividing the force needed to displace the filling material by the lateral area. The lateral area formula of tapered inverted cone (SL) was used to calculate the bonding area of the root canal filling material: $SL = \pi(R + r)h$, where $\pi = 3.14$, R is the mean radius of the coronal area of the slice in mm, r is the mean radius of the apical area of the slice in mm, and h is the height/thickness of the filling material.

Before the test, the height (h) of the slices was measured through a digital caliper (Digimess, Shiko Precision Gaging Ltd, China) and the perimeter and radius (major and minor) were measured by a stereo microscope (Leica, M165C, Leica Microsystems, Germany) using Las software v4.4 (Leica, M165C, Leica Microsystems, Germany) for the calculation of the adhered lateral area.

After the bond strength test, the slices were analyzed at 25× magnification to verify the failure mode, which was divided into five groups, as follows: (a) adhesive failure in the dentin – filling material dislodged from the dentin; (b) adhesive failure in the filling material – gutta percha dislodged from the sealer; (c) mixed, when both adhesive failures occurred; (d) dentin cohesive failure – dentin fractured; and (e) sealer cohesive failure – sealer fractured.

The second slice of each third was used for analysis of sealer penetrability into dentinal tubules according to the surface decalcification protocol (6 mol L^{-1} of hydrochloric acid), followed by deproteinization in 2.5% NaOCl. For the evaluation of gaps, the third slice of each sample was dehydrated in an ascending ethanol series. Both analyses were performed on the coronal face of the slices (coronal, middle, and apical thirds). All slices were covered with gold-palladium and examined under a scanning electron microscope (EvoMa10, Carl Zeiss, Munich, Germany) at 75×, 100× and 500× magnifications.[11]

Statistical analysis

The data were subjected to normality tests (Shapiro-Wilk) and homogeneity of variance (Levene's test). Bond strength data were analyzed by two-way ANOVA with a split-plot design and post-hoc Tukey's test ($\alpha = 0.05$) using the SAS 9.1 software (SAS, Cary, USA).

Results

Bond strength data are shown in Table 1.

There was a significant difference in irradiation ($p < 0.0001$), final irrigation solution ($p < 0.0001$), and root third ($p < 0.0001$). The interactions between irradiation and final irrigation solution influenced bond strength ($p = 0.0444$). However, there was no statistically significant difference in irradiation and root third ($p = 0.0900$), final irrigation solution and root third ($p = 0.8413$), and irradiation, final irrigation solution, and root third ($p = 0.5660$).

Table 1. Push-out bond strength means (in MPa) of the filling material to root dentin at the different root thirds in specimens subjected or not to radiation therapy with different final irrigation surface treatments.

Surface treatments	Non-irradiated			Irradiated		
	Coronal	Middle	Apical	Coronal	Middle	Apical
NaOCl	2.97 ± 0.86	2.37 ± 0.89	1.82 ±0.52	2.15 ± 0.57	1.75 ± 0.62	1.12 ± 0.61
EDTA	4.46 ± 1.58	4.41 ± 1.50	2.87 ± 1.04	2.49 ± 0.77	2.11 ± 0.86	1.82 ± 0.55
Chitosan	4.23 ± 1.70	3.57 ± 1.32	2.37 ± 1.07	2.73 ± 0.59	2.25 ± 0.70	2.14 ± 0.80
Pooled Average		3.27 ± 1.47^{a}			2.07 ± 0.79^{b}	

*Different letters indicate significant differences (ANOVA's $p < 0.0001$).

Table 2. Push-out bond strength means (in MPa) of the filling material to root dentin at the different root thirds.

Root third	Mean ± Standard deviation	
Coronal	3.17 ± 1.38	A
Middle	2.74 ± 1.36	B
Apical	2.09 ± 0.97	C

*Different letters indicate significant differences (Tukey's $p < 0.0001$).

Table 3. Push-out bond strength means (in MPa) of the filling material to root dentin for the different surface treatments in specimens subjected or not to radiation therapy.

Surface treatment	Irradiation	
	Non-irradiated	Irradiated
NaOCl	2.39 ± 0.89^{Ab}	1.68 ± 0.72^{Bb}
EDTA	3.92 ± 1.54^{Aa}	2.14 ± 0.77^{Bab}
Chitosan	3.51 ± 1.47^{Aa}	2.37 ± 0.73^{Ba}

*Different upper-case letters indicate statistical difference on the lines and different lower-case letters indicate statistical difference in the columns (Tukey's $p < 0.05$).

Irradiated teeth had lower bond strength values than non-irradiated teeth ($p < 0.0001$) (Table 1). Considering the interaction between irradiation and the final irrigation solution, Tukey's test (Table 2) showed that irradiation reduced bond strength in the final irrigation with NaOCl ($p = 0.0191$), EDTA ($p < 0.0001$), and chitosan ($p = 0.0003$). Regarding non-irradiated teeth, treatment with EDTA and chitosan showed the highest bond strength, with no statistical difference between them ($p = 0.1758$), and the NaOCl-treated teeth presented the lowest bond strength values ($p < 0.05$). For the irradiated teeth, the final irrigation with chitosan demonstrated higher bond strength than with NaOCl ($p = 0.0211$), and EDTA treatment presented similar results to the groups treated with chitosan ($p = 0.4391$) and NaOCl ($p = 0.1115$).

Regarding final irrigation protocols, Tukey's test showed that the use of EDTA and chitosan resulted in higher bond strength compared to the NaOCl group ($p < 0.001$), and that there was no statistical difference when compared to EDTA and chitosan ($p = 0.9080$) (Table 3).

The failure mode is presented in Table 4. For NaOCl, cohesive failures in the dentin increased after radiation at the cervical and middle thirds. For EDTA, there was an increase in adhesive failures in the dentin at all thirds after irradiation. For chitosan, there was an increase in adhesive failures in the filling material and a decrease in adhesive failures in the dentin at all thirds after irradiation.

The SEM analysis showed that, irrespective of the final irrigation solution used, irradiated specimens had a larger number of gaps at the dentin-filling material interface when compared to non-irradiated ones. Non-irradiated specimens treated with EDTA and chitosan showed greater homogeneity and adaptation of the filling material to the root dentin (Figure 1) when compared to non-irradiated teeth treated with NaOCl in all of the analyzed regions.

Moreover, the non-irradiated specimen treated with EDTA and chitosan showed few areas of short and interrupted resin tags, irregularly distributed and parallel to the peritubular dentin (Figure 2) when compared to irradiated specimens, which did not show resin tag formation. Regarding NaOCl-irrigated specimens, the presence of sealer tags was rarely observed, and when present, they were smaller, less numerous, and even more irregularly disposed compared to the EDTA and chitosan groups and were observed only in non-irradiated teeth.

Table 4. Distribution of failure modes (%) after the push-out test for the different surface treatments at different root thirds in specimens subjected or not to radiation therapy.

Failure mode	NaOCl						EDTA						Chitosan					
	Irradiated			Non-irradiated			Irradiated			Non-irradiated			Irradiated			Non-irradiated		
	C	M	A	C	M	A	C	M	A	C	M	A	C	M	A	C	M	A
Ad	0	30	10	30	10	40	20	20	10	10	0	0	0	10	20	50	30	40
Af	20	10	40	20	20	50	10	10	20	20	70	60	10	10	40	0	0	10
Ma	40	40	50	50	60	10	40	40	60	60	10	30	40	40	40	40	40	30
Cd	40	20	0	0	10	0	30	30	10	10	20	10	50	40	0	10	30	20
Cf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*C: coronal third; M: middle third; A: apical third. Ad: adhesive to dentin; Af: adhesive to filling material; Ma: mixed adhesive; Cd: cohesive in dentin and Cf: cohesive in filling material.

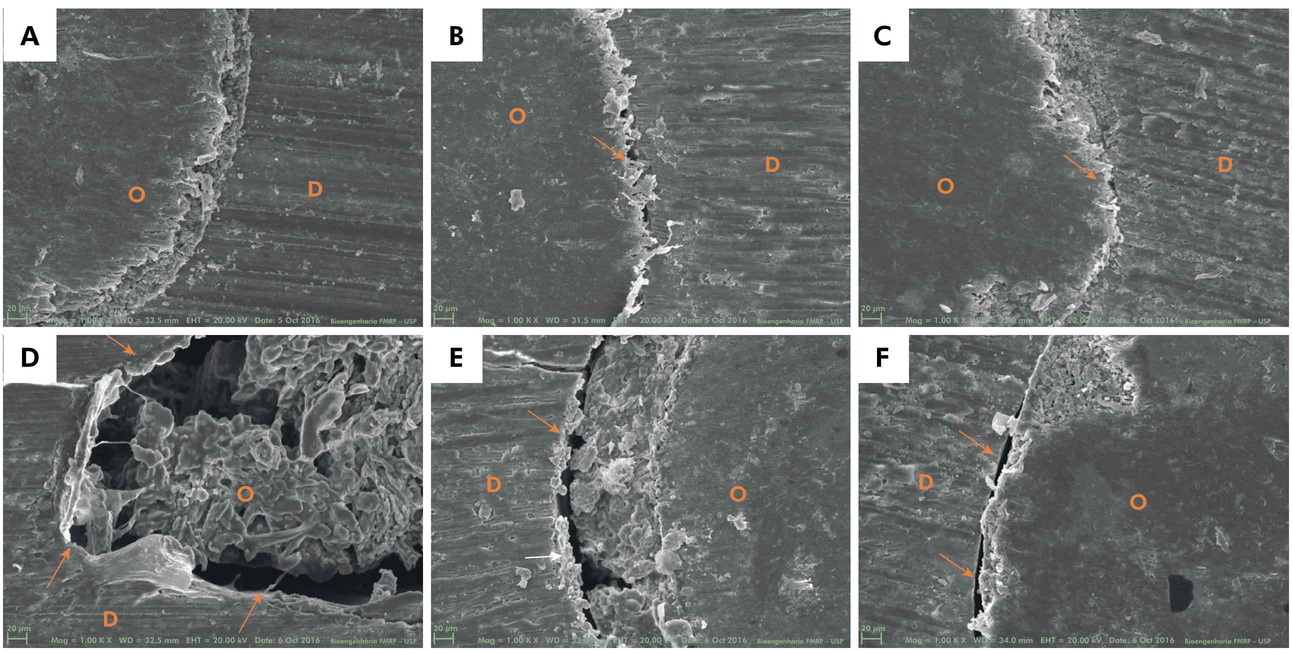

Figure 1. SEM micrographs obtained after the dehydration protocol, showing the sealer/dentin interface in non-irradiated and irradiated teeth, irrigated with (A) NaOCl, (B) EDTA, and (C) chitosan, and filled with the lateral condensation technique using AH Plus sealer (1000x). D: dentin; O: obturation.

Discussion

Although current protocols recommend fractionation of radiation for the repair of adjacent healthy tissues,[6,7] acute and late adverse events have been commonly observed following radiation therapy.[8,9,13]

Among late adverse effects, radiation caries has been shown to be highly prevalent due to the increased survival of these patients and changes in the dentin ultrastructure, causing severe destruction of enamel and dentin, with pulp alterations in most cases, determining the need for endodontic treatment.[19] Therefore, it is important to evaluate different protocols for the endodontic treatment of teeth that have been subjected to radiation therapy.

The present study aimed to simulate the radiation doses used to treat cancer patients subjected to fractionated doses of 2 Gy for 5 consecutive days with 30 cycles/6 weeks, totaling 60 Gy.[6,7] This protocol has been used to study the changes radiation therapy produces in the dental structure.[11,20,21]

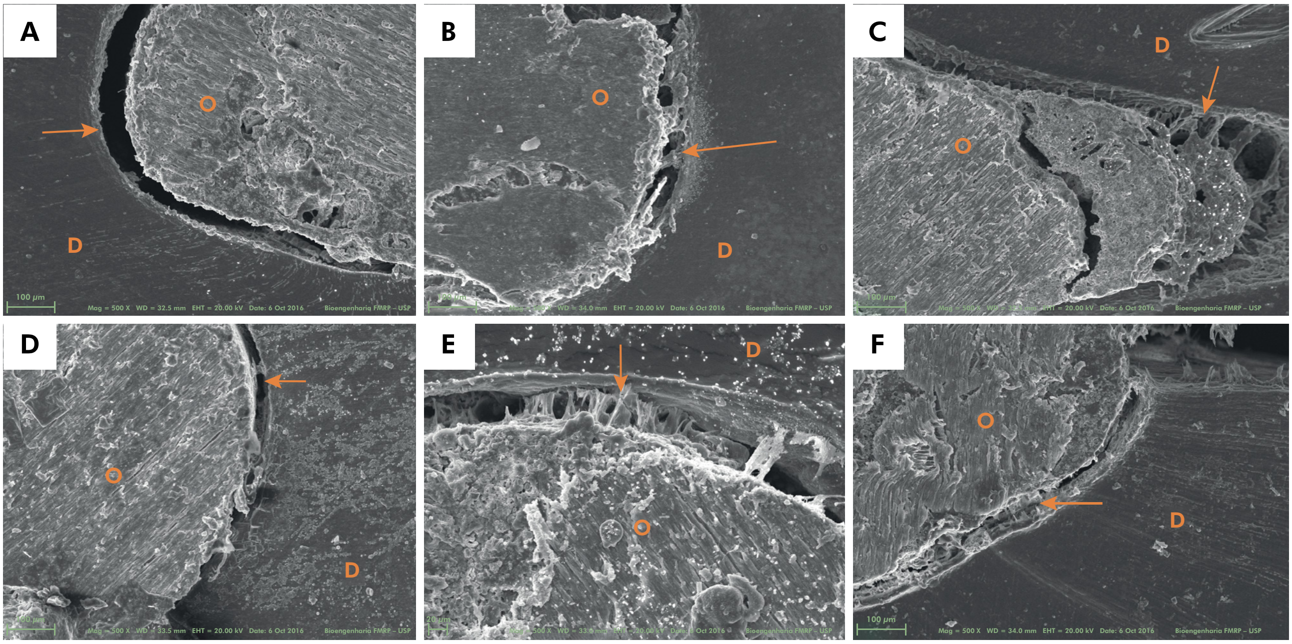

Figure 2. SEM micrographs obtained after the surface deproteinization protocol, showing the sealer/dentin interface in non-irradiated and irradiated teeth, irrigated with (A) NaOCl, (B) EDTA, and (C) chitosan, and filled with the lateral condensation technique using AH Plus sealer (1000x). D: dentin; O: obturation.

Another important factor was the use of artificial saliva for tooth storage, which, although not exactly reproducing exactly the qualities of natural saliva, mainly in cases of irradiated patients with altered salivary flow, secretion, and composition, is still the option that most closely resembles the clinical conditions of natural saliva in patients not subjected to radiation therapy.[11] Note that the samples were kept in distilled water during irradiation, since immersion in artificial saliva could hamper the homogeneous distribution of irradiation due to its viscosity and high ion concentration.[11,14] Furthermore, water is a major constituent of human tissue, thus the use of distilled water during radiation therapy can physically and chemically simulate the surrounding soft tissues by formation of free radicals.[11]

Teeth with amalgam restorations were excluded since, when in contact with irradiation, these restorations can increase the amount of secondary radiation,[11,22] which could hamper the control of variables and the standardization of results.[11]

In this study, regardless of the final irrigation solution and root third, irradiated teeth had lower bond strength than non-irradiated ones, which is probably associated with changes in the dentin ultrastructure such as obliteration of dentinal tubules,[14,23] alterations in the intertubular, peritubular, and intratubular dentin,[14] and with the fragmentation of the network of collagen fibers of the dentinal tissue and its deproteinization.[9,13,14,24] It is suggested that head and neck radiation therapy may lead to alterations in the amide III group present in the collagen structure and result in disorganization of the secondary structure of the protein unit that forms the collagen fibers,[25] modifying the natural arrangement between mineral and organic contents of dentin, changing its physical and mechanical properties.

In irradiated teeth, final irrigation with 0.2% chitosan showed higher bond strength compared to teeth irrigated with 1% NaOCl and 17% EDTA, which were statistically similar. Although chitosan has a similar chelating action to that of EDTA[16], teeth undergoing radiation therapy with deproteinization and defragmentation of the network of collagen fibers,[26] chitosan probably increased the resistance of collagen fibers to degradation by collagenase,[27] besides stabilizing the structure of collagen already compromised by irradiation.[18]

However, it is known that the use of 1% NaOCl alone does not promote smear layer removal, preventing the contact between endodontic sealers and intertubular dentin, which may justify the decreased bond strength found in this study.[28]

Dentin is characterized as a heterogeneous substrate due to its constitution, which has about 70% of inorganic material, 20% of organic material, and 10% of water,[29,30] wherein the organic material consists mainly of collagen fibers[31]. According to Soares et al.,[21] due to the fragmentation of the collagen fiber network, radiation has been shown to be more damaging to organic than to inorganic components, which may add to the explanation of the bond strength reduction of the AH Plus sealer after irradiation, regardless of the final irrigation solution used. The literature shows that hybridization is the primary process during the adhesion of hydrophobic resin materials to dentin and, in this process, most of the adhesion is promoted by micromechanical fixation of the collagen matrix inside of the intertubular dentin.[32,33]

Qualitative SEM analysis also evidenced the presence of gaps at the dentin-filling material interface more expressively in specimens treated with 17% EDTA and 0.2% chitosan after irradiation, corroborating the decrease of bond strength and an increase in the prevalence of adhesive failures in dentin and in the filling material, respectively. The interface integrity is crucial for the sealing, minimizing the chances of recontamination, which could induce to failure of endodontic treatment.[34] During the curing of resin materials, shrinkage stress increases and the root canal sealer tends to dislodge the sealer-dentin interface, forming gaps.[35]

Besides the influence of irradiation on the reduction of resin tag formation, probably due to obliteration of dentinal tubules[14,23] and to alterations in the intertubular, peritubular, and intratubular dentin at 60 Gy cumulative doses, short, asymmetric and low-density resin tags were observed in SEM when 17% EDTA and 0.2% chitosan were used.

Cohesive failure was also observed, independently of the final irrigation solution and root third, which may have occurred due to the association of the action of biomechanical preparation system, as reciprocating counterclockwise and clockwise motion can create areas of tension that can generate dentinal cracks,[36] and dentin ultrastructure alterations in irradiated teeth such as deproteinization, microhardness reduction, alterations in its crystal structure, fragmentation of collagen fibers, and obliteration of dentinal tubules.[12,13,14]

Considering that radiation therapy is associated with high caries prevalence, which may heighten the need for endodontic treatment, and that it is a commonly used treatment for head and neck cancer, the results presented herein open perspectives for new studies, such as demonstrating whether the use of filling materials is reproducible in different filling techniques, investigating more suitable techniques and materials, and developing new dentin surface treatment protocols to mitigate the harmful effects of irradiation on bond strength, consequently contributing for a better understanding and endodontic treatment plan for a more successful treatment of patients undergoing radiation therapy.

Conclusion

Radiation of teeth before root canal filling was correlated with lower push-out bond strength, regardless of the final irrigation solution used. In addition, the chitosan solution increased the bond strength of teeth subjected to radiation therapy when compared to teeth treated with NaOCl and EDTA.

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