The Adverse Effects of Radiotherapy on the Structure of Dental Hard Tissues and Longevity of Dental Restoration

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Abstract

Purpose:
The main goal of this study was to evaluate the impact of different ionizing radiation doses on the mineral (carbonate/phosphate ratio, crystallinity index [CI]) and organic (amide III/phosphate, amide I sub-band ratios) structures, as well as the microhardness, of enamel and dentin, along with their influence on the bonding strength stability of the etch-and-rinse (ER) and self-etch (SE) dental adhesive strategies.

Materials and methods:
Enamel and dentin human tissue specimens were irradiated (with 0, 20, 40, and 70 Gy radiation doses, respectively) and sectioned to perform an attenuated total reflection-Fourier transform IR spectroscopy assay (ATR-FTIR) and the Vickers microhardness (VHN) test to conduct a biochemical and biomechanical evaluation of the tissues. Regarding the adhesive properties, restored enamel and dentin specimens exposed to the same radiation doses were submitted to microshear bond strength (μSBS) tests for enamel in immediate time (IM) and to microtensile bond strength (μTBS) tests after for IM and 12-month (12 M) period of time, Mann–Whitney U tests were implemented, using the ATR-FTIR data for significant differences (α < 0.05), and three- and two-way analyses of variance, along with post-testing, were performed on the μTBS and μSBS data (MPa), respectively (Tukey post hoc test at α = 0.05).

Results:
The ATR-FTIR results showed a significant decrease (p <.05) in the amide III/phosphate ratio after 20 Gy for the enamel and after 40 Gy for the dentin. The CI was significantly reduced for both tissues after a dose of 70 Gy (p <.05). All radiation doses significantly decreased microhardness values, relative to the respective enamel and dentin controls (p <.05). In both tissues and adhesive strategies, the decrease in bond strength was influenced by ionizing radiation starting from 40 Gy. The ER strategy showed high percentages of enamel cohesive failure. In general, ER in both tissues showed greater and more stable bond strength than SE against increased radiation doses and long term.

Conclusions:
It is possible to conclude that structural alterations of enamel and dentin are generated by all radiation doses, decreasing the microhardness of dental hard tissues and influencing bond strength over time, starting at 40 Gy radiation dose. The etch-and-rinse strategy demonstrates better adhesive performance but generates cohesive fractures in the enamel.
Introduction
Head and neck cancers represent 4% of cancer incidences worldwide and cause 360,000 deaths annually (Bray et al. [4]). Radiotherapy is one of the most effective options for treatment (Budach and Tinhofer [6]). However, the high-energy radiation involved in this treatment produces side effects in the tissues surrounding the tumor (Moding et al. [19]). These side effects include clinical complications in the structure of the teeth (e.g. cracks, delamination) and aggressive caries processes that consequently need intervention and effective restorative treatments (González-Arriagada et al. [14]).

Adhesive dental restorations are the main treatment to replace lost dental hard tissues, due to caries and non-curious lesions. The adhesive interface formed between the tooth and restoration shows bonding failures over time (Breschi et al. [5]; Bedran-Russo et al. [3]). The occurrence of these failures increases the likelihood of oral biofilm accumulation, infections, and tooth loss, which can lead to catastrophic complications for irradiated patients, such as those suffering from osteoradionecrosis (Niewald et al. [22]). Consequently, the adhesive restoration treatment and its maintenance before and during radiotherapy are recommended (González-Arriagada et al. [14]). However, there is a high need for post-radiotherapy restorative treatments among these patients, and the indication of the most stable adhesive strategy to be applied on irradiated dental hard tissues has not yet been thoroughly characterized.

Enamel and dentin have different concentrations of organic and mineral phases and water content (Reyes-Gasga et al. [27]), giving them distinctive physical–mechanical characteristics, which, when integrated, allow for the functional stability of the tooth against oral challenges (Tobe et al. [34]). To perform a dental restoration, the composition and method of application of the adhesive systems should be adapted to the structural characteristics of enamel and dentin. Thus, it is plausible that damages to the structure of these tissues can influence adhesive performance; however, there are no consensus effects if irradiation to the organic and mineral phase (Madrid Troconis et al. [17]) on associated with the immediate bond strength results (within 24 hours) and don’t have studies that performed a long-term evaluation. In addition, existing studies (Aggarwal [1]; Dibo da Cruz et al. [11]; Naves et al. [20]; Goncalves et al. [13]; Madrid et al. [16]; Tobe et al. [34]) present methodological differences in the storage of the teeth used, which could induce variations in the mechanical, chemical, and biological responses of in vitro studies.

Adhesive dentistry refers in micromechanical interlocking of the infiltrated adhesive system in the microspaces obtained in enamel and the collagen fibrils exposed in dentin by etching, forming the hybrid layer (Breschi et al. [5]; Pashley et al. [26]; Bedran-Russo et al. [3]). Its stability depends on the strategy used to condition these tissues, the infiltration capacity of the monomers of adhesives systems, and the conformation of the polymer network, among other factors (Breschi et al. [5]). To achieve hybridization, the etch-and-rinse (ER) and self-etch (SE) adhesive strategies through phosphoric acid (30%–40%) or acidic monomers (incorporated in the adhesive system), respectively, carry out the demineralization of the tissue necessary for the infiltration of the substrate by monomers (8). The effectiveness of the adhesives strategies in the different radiation doses and the association of
chemical factors of dental hard tissues with the adhesive interface stability over time are unknown until now.

Thus, the main goal of this study was to evaluate the effects of ionizing radiation doses on the mineral (carbonate/phosphate ratio, crystallinity index) and organic (amide III/phosphate, CH2/amide III) structure and microhardness of enamel and dentin, as well as their influence on the stability of the bond strength of SE and ER adhesive strategies. The following null hypotheses were tested in this study: (1) There are no structural (mineral and organic) and microhardness changes in dentin and enamel at various radiation doses (20, 40, 70 Gy) when compared to their respective sound tissues; (2) the adhesive strategies (ER and SE) applied to irradiated enamel (20, 40, 70 Gy) when compared to sound enamel do not affect the immediate bond strength; and (3) the adhesive strategies (ER and SE) applied to irradiated dentin (20, 40, 70 Gy) when compared to sound dentin do not affect the immediate and 12-month bond strength.

Materials and methods

Tooth selection and experimental design

A total of 52 extracted, caries-free human third molars were collected after obtaining the patient's informed consent (local Ethics Committee Review Board #CEC125-16). The teeth were extracted and immediately frozen to −20 °C. Before 24–48 h, they were defrosted in room temperature in distilled water for 90 min and irradiated immediately by a Cobalt irradiation unit at 0, 20, 40 and 70 Gy radiation doses (de Barros da Cunha et al. [10]). For each test, the teeth were assigned to randomized blocks, using computer-generated tables.

Twelve teeth (12) were cut on the axial axis in the buccolingual direction (ISOMET 1000, Buehler Ltd., Lake Bluff, IL, U.S.A.), to obtain two slabs (1 mm thickness). One slab per tooth was assigned to the chemical analysis, using attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), and the other slab was subjected to the Vickers microhardness test. The internal surfaces of each slab were polished with wet sandpaper (#1500–3000 grit SiC for 60 s) and ultrasonically washed in distilled water (60 s) between each polishing.

Forty teeth (40) were assigned to the bond strength tests. For each tooth, the enamel faces (buccal, lingual, and proximal) were sectioned to obtain flat surfaces for the microshear bond strength (μSBS) tests. Then, the exposed occlusal dentin surfaces were used for the microtensile bond strength (μTBS). After cutting, the enamel and dentin surfaces were standardized with wet sandpaper (#600-grit SiC for 60 s) and ultrasonically washed in distilled water (60 s).

ATR-FTIR chemical analysis

The analytical method of ATR-FTIR spectroscopy assay was performed on tooth slabs (n = 3) of different radiation doses groups (0, 20, 40, and 70 Gy). Three spectra were obtained from each specimen after application of ionizing radiation treatment, as described above, employing an FTIR spectrometer (Nicolet i55, Thermo-Nicolet Instruments, Madison, WI, U.S.A.). The spectra were collected between 4000 and 650 cm\(^{-1}\), at a spectral resolution of 4 cm\(^{-1}\), with a diamond crystal (Smart Orbit, Thermo Fisher Scientific, Waltham, MA, U.S.A.), using the co-addition of 64 scans. The absorption band characteristic of peptide bonds from amides I, II, and III for collagen components,
carbonate, and phosphate bands was considered, and to perform a semi-quantitative comparison among groups, the background signal was subtracted. The areas under the peaks were analyzed after normalization (Chang and Tanaka [9]; Reyes-Gasga et al. [27]; Paschalis et al. [25]). For the crystallinity index analysis, the calculation was performed according to the equation: \( IC = \frac{I_{551} + I_{597}}{I_{588}} \), where \( I_{551} \), \( I_{597} \), and \( I_{588} \) represent the intensities of 551-, 597-, and 588-cm\(^{-1}\) bands, respectively (Severcan [29]; Wang et al. [35]; Reyes-Gasga et al. [27]; Thompson et al. [33]; Tobe et al. [34]). OMINIC (Thermo Fisher Scientific, Waltham, MA, U.S.A.) software was used for the normalization of spectra and the analyses (Table 1).

**Table 1. General band assignment for the ATR-FTIR spectra of dental hard tissue.**

<table>
<thead>
<tr>
<th>Peak wavenumber (cm(^{-1}))</th>
<th>Name</th>
<th>Definition of the assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1680–1600</td>
<td>Amide I</td>
<td>Protein C = O stretching of protein, Type I collagen</td>
</tr>
<tr>
<td>1480–1580</td>
<td>Amide II</td>
<td>Protein N-H bend coupled with C-N stretch, Type I collagen</td>
</tr>
<tr>
<td>1200–1300</td>
<td>Amide III</td>
<td>Protein N-H bend coupled with C-N stretch, Type I collagen</td>
</tr>
<tr>
<td>1400–1580</td>
<td>( v^3 ) CO(_3^2^-)</td>
<td>Vibration mode of carbonate</td>
</tr>
<tr>
<td>963</td>
<td>PO(_4^3^-) ( v^1 )</td>
<td>Vibration mode of phosphate band</td>
</tr>
<tr>
<td>602</td>
<td>PO(_4^3^-) ( v^2 )</td>
<td>Vibration mode of phosphate band</td>
</tr>
<tr>
<td>1033</td>
<td>PO(_4^3^-) ( v^3 )</td>
<td>Vibration mode of phosphate band</td>
</tr>
<tr>
<td>562</td>
<td>PO(_4^3^-) ( v^3 )</td>
<td>Vibration mode of phosphate band</td>
</tr>
</tbody>
</table>

Enamel and dentin microhardness evaluation
The microhardness surface values were obtained with a microhardness tester (Shimadzu HMOV2000, Shimadzu Corporation, Kyoto, Japan). The Vickers hardness number (VHN) was determined by fitting a 100 kgf (enamel) and 50 kgf (dentin) load into the diamond indenter, which was then allowed on the surface for 30 s. Each slab per group (\( n = 3 \)), according to the radiation doses described, was evaluated at two sites that were directly adjacent to the portion of the occlusal cusp dentin–enamel junction (DEJ). In both the enamel and the dentin, the sites were positioned 50 \( \mu \)m away from the DEJ. An average of six indentations for each area was calculated; each indentation was made 100 \( \mu \)m apart to minimize the interactions between neighboring brands.

Resin bond strength test to enamel and dentin
The resin bond strength tests (\( \mu \)SBS, \( \mu \)TBS), the enamel (\( n = 5 \)), and dentin (\( n = 5 \)) specimens were randomly distributed among into eight groups, according to the combination of the independent variables: adhesive strategy (2-step etch-and-rinse [ER] or 1-step self-etch [SE]) and radiation dose (control 0 and experimental 20, 40, and 70 Gy). In all bond strength groups, the adhesive system Scotch Bond Universal (SBU; 3 M ESPE, St Paul, MN, U.S.A.) was applied following to the SE or ER strategy, in accordance with the manufacturer’s instructions (Table 2). All teeth received a nanofilled resin composite restoration (Filtek Z350, 3 M ESPE, St Paul, MN, U.S.A.), light-polymerized using an LED light-curing unit set at 800 mW/cm\(^2\) (Bluephase N MC, Ivoclar Vivadent, Schaan, Liechtenstein).
Table 2. Materials (batch number), composition, and application mode.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition</th>
<th>Adhesive strategy</th>
<th>Adhesive strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek Z350 (6028A2B)</td>
<td>Bis-GMA, UDMA, TEGDMA, Bis-EMA, silanated silica, silanated zirconia, photoinitiators</td>
<td>Self-etch</td>
<td>Etch-and-rinse</td>
</tr>
<tr>
<td>Scotchbond Universal Adhesive – SBU (70919A)</td>
<td>1. Etchant: 32% phosphoric acid, water, synthetic amorphous silica, polyethylene glycol, aluminum oxide (Scotchbond Universal Etchant). 2. Adhesive: MDP Phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkeinoic acid copolymer, filler, ethanol, water, initiators, and silane.</td>
<td>1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely. 3. Light-cure for 10 s.</td>
<td>1. Apply etchant for 15 s. 2. Rinse for 10 s. 3. Air dry 5 s. 4. Apply adhesive as in the self-etch strategy.</td>
</tr>
</tbody>
</table>

Bis-GMA: bisphenol glycidyl methacrylate; UDMA: urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; Bis-EMA: ethoxylated bisphenol-A dimethacrylate; MDP: methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate.
To perform the microshear bond strength test on enamel substrate, the specimens were preparing following a previously published μSBS protocol (Shimaoka et al. [31]). A thin wire (0.2 mm diameter) was looped around the base of each resin composite specimen, making contact with half of its circumference, always keeping the setup (the resin–enamel interface, the wire loop, and the center of the load cell) in alignment to ensure correct orientation of the shear forces. A shear load was applied at a crosshead of 1 mm/min until failure. The μSBS values were calculated by dividing the load at failure by the surface area (mm²) to determine the μSBS in megapascals (MPa). The failure mode was classified as cohesive (C; failure exclusively within dentin or resin composite), adhesive (A; failure at the resin–dentin interface), or mixed (M; failure at the resin–dentin interface that included cohesive failure of the neighboring substrates). The failure mode analysis was performed under a stereomicroscope at 10× magnification (Model SZ-PT, Olympus, Tokyo, Japan).

On the dentin substrate, the specimens for the μTBS test were performed following a previously published protocol (Sezinando et al. [30]). Half of the resin–dentin bonded specimens were submitted under tension until fracture at 0.5 mm/min (23 5S, Emic Instron, Sao Jose dos Pinhais, PR, Brazil) immediately (24 hours), and the other half were stored in distilled water at 37 °C and tested in the same way after 12 months. The μTBS was calculated by dividing the load at failure by the cross-sectional bonding area. The failure mode was classified in the same way as it was for enamel, previously described.

Statistical analysis
The ATR-FTIR data were statistically analyzed using a Mann–Whitney U test for significant differences (α < 0.05), according the different radiation doses groups (Gy). The μTBS data (MPa) were subjected to a three-way ANOVA analysis of variance (radiation dose vs. adhesive strategies vs. storage time), the μSBS data (MPa) to a two-way analysis of variance (radiation dose vs. adhesive strategies), and both to a post hoc test (Tukey post hoc test at α = 0.05) for pairwise comparisons.

Results
Structural composition of enamel and dentin
The ATR-FTIR spectroscopy assay of human dentin specimens revealed absorption bands, considered for collagen-associated peaks (Chang and Tanaka [9]; Cakmak et al. [8]) and apatite-associated peaks (Reyes-Gasga et al. [27]; Thompson et al. [33]). The analyzed bands are shown in Table 1.

The series of bands ranging from 800–1580 cm⁻¹ can be attributed to the presence of carbonate groups, and those between 560 and 1040 to the phosphate components; the bands were used as a means for quantitative evaluation of their presence under the different radiation dose cycles, as shown in Figure 1. The analysis of the second derivate was used to evaluate the presence and position of infrared bands for each component described, as suggested by the literature (Figure 2).
Figure 1. FT-IR spectrum of different irradiation doses stage (0, 20, 40, and 70 Gy), to enamel tissue in (A) and dentin in (B). In the graphics possible identifying the intensity band to amide I (1), amide II (2), CH2 and CO3 (3), and the phosphate phases: PO4 v3 (4), and PO4 v1 (5).

Figure 2. The graphics shown, the ratios resulted for Cl, protein/mineral phases and amide I sub-bands of enamel tissue in (A–D), and to dentin tissue in (E–H) graphics respectively, under different ionizing radiation doses were applied sob dentin tissue. Identical letters are statistically similar (p >.05).

The qualitative analyses of the ATR-FTIR spectra obtained reveal visible molecular changes in organic content and decreased intensity of the amides II and III bands, correlating with the intensification of the radiation dose. Similar results were observed in non-organic content, which showed decreased intensity of the CO3^- and PO4^-3 bands, attributable to the effect of radiation idem (Figures 1 and 2).

Enamel and dentin microhardness evaluation

The results (Figure 3) indicated a significant decrease in microhardness for all radiation doses, relative to the respective enamel and dentin controls (p <.05). The average microhardness of enamel is greater than that of dentin (p <.05). There were non-statistical difference in dentin irradiated with doses of 20, 40, and 70 Gy (p >.05). The microhardness of enamel at 70 Gy is greater than 20 Gy (p <.05), but does not differ from its microhardness at 40 Gy (p >.05).
Figure 3. The graphic shown, the values resulted for VHM analyses to enamel (black) and dentin (gray) tissues, under different ionizing radiation doses (0, 20, 40, and 70 Gy). Identical letters are statistically similar (p >.05).

Resin bond strength test to enamel and dentin
The frequency and percentage of each fracture pattern mode and premature failure are shown in Table 3. Regarding the fracture pattern for the SE-treated dentin groups and enamel, most of the specimens showed adhesive or adhesive/mixed failures. For the enamel ER strategy, an increase in cohesive failures was observed at 20 Gy (40.5% on average), 40 Gy (44.4%), and 70 Gy (52.6%). On enamel treated by the SE and ER strategies, a significant μSBS decrease was shown at 40 Gy, compared to the respective controls and 20 Gy (p <.05). For SE, there was a significant decrease at 70 Gy, with respect to all dose groups (p <.05). For ER, there were no observable differences between 40 and 70 Gy (p >.05) (Table 4).
Table 3. Number (%) of specimens according to fracture mode for the experimental groups.

<table>
<thead>
<tr>
<th>Adhesive strategies</th>
<th>Radiation dose</th>
<th>Fracture pattern mode</th>
<th>Dentin</th>
<th>Enamel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>C</td>
<td>M</td>
</tr>
<tr>
<td>SE</td>
<td>0 Gy</td>
<td>21 (60.1)</td>
<td>3 (8.5)</td>
<td>9 (25.7)</td>
</tr>
<tr>
<td>20 Gy</td>
<td>24 (70.6)</td>
<td>2 (5.9)</td>
<td>6 (17.6)</td>
<td>2 (5.9)</td>
</tr>
<tr>
<td>40 Gy</td>
<td>27 (75.0)</td>
<td>2 (5.6)</td>
<td>7 (19.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>70 Gy</td>
<td>23 (62.2)</td>
<td>6 (16.2)</td>
<td>6 (16.2)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>ER</td>
<td>0 Gy</td>
<td>26 (76.5)</td>
<td>3 (8.8)</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>20 Gy</td>
<td>17 (50)</td>
<td>6 (17.6)</td>
<td>10 (29.4)</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>40 Gy</td>
<td>16 (44.4)</td>
<td>7 (19.4)</td>
<td>13 (36.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>70 Gy</td>
<td>25 (73.5)</td>
<td>6 (17.6)</td>
<td>2 (5.9)</td>
<td>1 (3.0)</td>
</tr>
</tbody>
</table>

Table 4. Microshear bond strength (μSBS) values (means ± standard deviations in MPa) of the different experimental groups* [3].

<table>
<thead>
<tr>
<th>Radiation dose</th>
<th>Adhesive strategies</th>
<th>0 Gy</th>
<th>20 Gy</th>
<th>40 Gy</th>
<th>70 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SE</td>
<td>22.31 ± 1.5 A,b</td>
<td>21.68 ± 1.2 A,b</td>
<td>18.44 ± 1.1 B,b</td>
<td>16.27 ± 1.6 C,b</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>27.38 ± 1.7 A,a</td>
<td>25.96 ± 1.0 A,a</td>
<td>21.89 ± 2.1 B,a</td>
<td>21.66 ± 1.2 B,a</td>
</tr>
</tbody>
</table>

Comparisons are valid only within of same rows (capital letter) or columns (lower case). Means identified with the identical letters are statistically similar ($p > .05$).

Dentin in the immediate time, for SE, the first significant decrease occurred at 40 Gy, compared with its control ($p = .0015$), which did not reflect differences from its condition at 20 Gy ($p = .085$). A significant decrease was observed at 70 Gy with respect to all SE groups ($p < .05$). However, in the same time with the ER strategy, a statistically significant decrease only presented at 70 Gy compared to the other ER group’s (0, 20, and 40 Gy) ($p < .05$) (Table 5). After 12 months, in the dentin substrate for both adhesive strategies in general (SE and ER) and all radiation doses (20, 40, and 70 Gy), there was a significant μTBS decrease when compared to the immediate time ($p < .05$), with exception of SE at 70 Gy (Table 5). For SE strategy, no differences were observed between 40 and 70 Gy ($p = .05$), but a significant decrease of the μTBS was presented, relative to the non-irradiated control and 20 Gy ($p < .05$). Finally, with the ER strategy the non-irradiated control and 20 Gy manifested no significant differences ($p = .556$), but at 40 and 70 Gy, the μTBS decreased, compared to them ($p < .05$) after 12 months (Table 5).

Table 5. Microtensile bond strength (μTBS) values (means ± standard deviations in MPa) of the different experimental groups* [4].

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Adhesive strategies</th>
<th>Self-etch</th>
<th>Etch-and-rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate (24 hours)</td>
<td>0 Gy</td>
<td>42.37 ± 6.6 A,a</td>
<td>34.67 ± 4.3 AB,a</td>
</tr>
<tr>
<td></td>
<td>20 Gy</td>
<td>38.67 ± 8.2 B,a</td>
<td>34.91 ± 7.5 C,a</td>
</tr>
<tr>
<td></td>
<td>40 Gy</td>
<td>27.65 ± 4.2 A,a</td>
<td>46.01 ± 3.1 A,a</td>
</tr>
<tr>
<td></td>
<td>70 Gy</td>
<td>43.77 ± 5.9 AB,a</td>
<td>39.61 ± 5.5 B,a</td>
</tr>
<tr>
<td>Longevity (12 months)</td>
<td>0 Gy</td>
<td>31.16 ± 7.3 B,b</td>
<td>31.55 ± 4.9 B,b</td>
</tr>
<tr>
<td></td>
<td>20 Gy</td>
<td>31.55 ± 6.6 E,b</td>
<td>23.38 ± 4.9 DE,a</td>
</tr>
<tr>
<td></td>
<td>40 Gy</td>
<td>25.43 ± 8.6 A,b</td>
<td>38.61 ± 8.3 BC,b</td>
</tr>
<tr>
<td></td>
<td>70 Gy</td>
<td>36.49 ± 8.3 CD,b</td>
<td>29.16 ± 6.8 CD,b</td>
</tr>
</tbody>
</table>

Comparisons are valid only within of same rows (capital letter) or columns (lower case). Means identified with the identical letters are statistically similar ($p > .05$).

Discussion

The stability of the adhesive interface established between dental hard tissues and dental restoration determines the longevity of this treatment (Breschi et al. [5]). Therefore, the objective was to analyze the association between specific chemical changes and microhardness of irradiated dental hard tissues and their impact on the stability of the bond strength of dental restorations over time. The results of this study show that ionizing radiation damage dental hard tissues at all applied doses thereby rejected the first null hypothesis. The resin bond strength to dentin and enamel were not affected, provided that the bonding procedure was performed at low-ionizing radiation doses (20 Gy). However, the effects of the other doses of ionizing radiation on adhesive performance varied in accordance with
time and adhesive strategies. Thus, the second and third null hypotheses are partially rejected. This is the first study to show the association of the damages generated by the doses of ionizing radiation in the dental hard tissues with the longevity of the bond strength in the different adhesive strategies, whose antecedents in the existing literature are controversial (Madrid Troconis et al. [17]).

Radiotherapy is an effective and safe therapeutic method that consists in cumulative fractionated X-ray doses (1.8 or 2 Gy per session) delivered according to the understanding of the effects of cumulative tumor cell biology, its microenvironment, and the organism where it originated (Moding et al. [19]). Considering that these cellular and biological factors are not present in vitro radiation studies of extracted teeth, total doses were applied in a single session (Dibo da Cruz et al. [11]; de Barros da Cunha et al. [10]).

On dentin, in the organic content, which corresponds to collagen structure, an increase in the amide/PO₄ ratio at doses of 40 Gy was observed. Studies have demonstrated that even indirect effects of ionizing radiation can induce radiolysis in similar doses and elevate the concentration of advanced glycation end-products (AGEs), producing an excessive amount and non-enzymatic cross-link of collagen (Nguyen et al. [21]; Tobe et al. [34]). When the radiation doses were increased to a dose of 70 Gy, a dramatic decrease in the intensity of the same band was observed; accumulative high radiation doses may generate considerable damage to the collagen matrix (Maslennikova et al. [18]), thereby modifying the molecular structure of dentin tissue (Palmier et al. [24]; Lu et al. [15]), due to its susceptibility to the free radicals produced by the ejection of electrons, forming direct organic-free radicals (R•), and the ionization of H₂O molecules, leading to the formation of hydroxyl radicals (•OH) (Ronai and Benko [28]). It should be noted that free radicals have been associated with the inhibition of the polymerization reaction of adhesive systems, which negatively affects the stability of the bond strength to dental hard tissues thereof.

In concurrence, the 1660/1690 cm⁻¹ sub-band ratio, an amide I constituent, also increased as well at a 40 Gy dose of radiation. In previous studies, these sub-bands correspond to the ratio of non-reducible/reducible collagen cross-links in bone, skin, and dental hard tissues (Paschalis et al. [25]; Lu et al. [15]). Further, other studies based on collagen denaturation experiments demonstrated that the sub-band is representative of triple helices and 'free' carbonyl groups. These facts suggested that an apparent increase in the cross-link of the dentin collagen matrix was attributable to the effect of radiation at doses of 40 Gy (Bachmann et al. [2]; Paschalis et al. [25]; Lu et al. [15]).

The intensity of bands observed on the enamel spectra, corresponding to collagen structure and non-collagenous proteins, was lower than in the dentin, reflecting the poor organic content on this tissue (~1.5 wt.%) and to the high content of the inorganic matrix (~97 wt.%), composed mainly of carbonated hydroxyapatite (Bachmann et al. [2]). As such, the infrared bands corresponding to the amides have lower intensities, relative to the phosphate and carbonate bands, which makes it difficult to quantify the ratio of low-intensity amide III, as reported in other research (Reyes-Gasga et al. [27]; Lu et al. [15]). In addition, the ratio tended to decrease in accordance with the incremental increase of the radiation dose and the significant reduction from 20 Gy made it possible to observe the differences engendered by each radiation dose (40 and 70 Gy), as in dentin tissue. The self-deconvolution highlights the overlapped amide I sub-bands in the enamel under the different radiation doses (20, 40, and 70 Gy) (thus, it was possible to observe the same behavior at the 1660/1690 cm⁻¹ ratio as was
explained for the dentin tissue in this study). Due to the amide's I complex contours along, with the coupling of heterogeneous stretching modes of carbonyl groups, different sub-bands manifested in their region; this was necessary to perform the self-deconvolution and, in turn, observe the different vibrations (Doyle et al. [12]; Paschalis et al. [25]).

The ionizing radiation generated similar effects for non-organic content on dentin and enamel. The intensities and ratios of carbonates and phosphate phases apparently render changes in the hydroxyapatite crystal and lattice structure, starting at a dose of 40 Gy, and decreasing the crystallinity index of both tissues at high doses of gamma radiation (70 Gy). The crystallinity index obtained by the ATR-FTIR test is generally associated with the degree of geometrical deformation of the molecular and atomic unions inside the apatite structure (Reyes-Gasga et al. [27]). Thus, the reduction of the crystallinity may be interpreted in terms of lost bond symmetry for both tissues, when submitted to high gamma radiation doses (Lu et al. [15]).

This is the first in vitro study that seeks to document the longevity (12 months) of bond strength to the irradiated coronal dentin and establish the influence of different doses of radiation on its stability. All groups showed decreased dentin bond strength after the aging challenge (water storage); however, not all the adhesive strategies were influenced by radiation doses. The results showed no different dentin bond strength values for both adhesive strategies until doses of 20 Gy in the immediate time. After 12 months, this dose demonstrated similar aging behavior to that of the non-irradiated groups on dentin bond strength. These findings suggest that the formation of a hybrid layer over irradiated tissue does not affect the bond strength up to 20 Gy and may be equivalent to an adhesive interface formed over sound dental tissues prior to ionizing radiation (Bulucu et al. [7]; Aggarwal [1]; Dibo da Cruz et al. [11]; Naves et al. [20]; Yadav and Yadav [36]). In the immediate time of evaluation (24 h), similar results have been reported when the same low doses of radiation are applied (de Barros da Cunha et al. [10]). However, controversial results were observed as doses were increased (Naves et al. [20]), confirming the importance of performing the dental adhesive restorations before the dose increase. Although chemical changes associated with radiation at a dose of 20 Gy were observed in the dentin and enamel, these findings suggest that such changes were not sufficient to alter the behavior of the adhesive interface. In this way, the damage in bond strength presented at 0 and 20 Gy after 12 months may be associated with known factors of polymer degradation (Breschi et al. [5]) and endogenous activity (Bedran-Russo et al. [3]).

From doses of 40 Gy and higher, the dentin bond strength reflected a detrimental effect associated with ionizing radiation for both adhesive strategies. It is important to highlight that FTIR findings showed damages at high doses to the structure of the collagen fibrils; this may have hindered a homogeneous and stable hybridization of the adhesive system. Moreover, the polymer matrix of the adhesive system may be more susceptible to hydrolytic degradation, due to interaction with the free radicals in consequence of the ionizing radiation (Ronai and Benko [28]) results on the dentin, due to inhibition of the polymerization reaction by the high water content. Concerning the immediate time of the ER strategy, it was less affected than the SE when the doses increased from 40 to 70 Gy demonstrating also greater dentin bond strength than the SE after 12 months. It is possible that the deeper micromechanical interlocking and a thicker hybrid layer from the ER system (Pashley et al. [26]) provide greater retention. Given the assessment of both adhesive strategies after 12 months, it is
important to note that, at 40 Gy dose, the dentin bond strength was equivalent to the obtained at 70 Gy. Thus, the increased radiation dose did not raise its deterioration, probably on the account of the chemical bond of specific monomers of the adhesive (10-methacryloyloxydecyl dihydrogen phosphate and polyalkenoic acid copolymer) with the minerals of the tissue and the smear layer (39). In the case of the SE, that chemical bond increases the adhesive interface's mechanical properties, resistance to degradation, and longevity (Yoshihara et al. [40], [39]; Nurrohman et al. [23]; Yoshida et al. [38]; Sezinando et al. [30]).

In all doses applied in enamel, the ER strategy yielded greater bond strength than the SE-based strategy, due to the well-established mechanical interlocking of the polymerized adhesive in the interprismatic spaces, generated by the etching with phosphoric acid. However, when the microshear test was performed, a high percentage of enamel-cohesive fractures (40.5 to 52.6%) were observed, and only in the irradiated ER groups. Alterations in the organic enamel matrix were observed at 20 Gy doses, which, together with the interprismatic spaces (Madrid et al. [16]) consequent to the ionizing radiation, may have a direct impact on the changed degree of anisotropy reflected in the fracture resistance and reduced ability of the enamel to prevent cracks from spreading (Yahyazadehfar and Arola [37]), as well as in the drastic decrease observed in the microhardness. In addition, in the ER strategy, the application of phosphoric acid has an unspecified action, because it removes minerals and affects the organic content without discrimination, and its interaction with the irradiated enamel has not been clarified. Tabata et al. ([32]) attribute to etching, with 40% phosphoric acid, the deterioration of the integrity of the enamel adjacent to the restoration bonded with a two-step self-etching adhesive (pH = 2.1) and the formation of cracks. The superficial interaction and less depth etching of SE may account for the lower percentage of cohesive fractures over weakened enamel, even after ionizing radiation.

The enamel given a radiation dose of 20 Gy suffered a dramatic decrease in microhardness, consistent with a previous report that used the same measurement distance to the DEJ (Lu et al. [15]). It is possible that lower microhardness results cannot be observed at 40 and 70 Gy doses because mechanisms such as cross-links in the collagen matrix (induced by 40 Gy radiation), as shown in this study, and the glycation process (at high radiation doses), as exposed in the previous reports (Tobe et al. [34]), may have impacted the results. Other studies evaluating irradiated enamel, at 50 μm (Goncalves et al. [13]) and 150 μm (de Barros da Cunha et al. [10]) from the DEJ, showed neither harmful nor mild effects, regardless of the radiation dose applied. However, it is unclear the measurement area, the method of storing the teeth involved keeping them at a temperature of ~4 °C for up to 1 month after the extraction (Goncalves et al. [13]); these make a direct comparison with the results of previous studies difficult and points to a need to standardize methodologies for future studies. The microhardness analysis of the dentin at 20 Gy revealed similar effects to those of the enamel, with a significant decrease, and this condition remained stable until 70 Gy. There is still controversy over the results in the literature (Madrid Troconis et al. [17]) possibly because they involve comparing different depths of the irradiated dentin and regions of the tooth are compared, and also due to storage differences that, as indicated above, may generate alterations in the tissues.

Under a storage model for keeping the characteristics of a tooth as fresh as possible, our results demonstrate that all radiation doses affect the mineral and organic structures of enamel and dentin
accompanied by reduced microhardness, which could contribute to the advancement of the aggressive processes of caries and delamination of the tissue suffered by irradiated patients. Those structural changes are sufficient to affect the dentin and enamel bond strength from 40 Gy, showing advantages in the stability over time of the etch-and-rinse strategy. Therefore, these results could be considered a contribution in the selection of restorative treatment of post-radiotherapy patients.

Conclusion

Based on the chemical, mechanical, and adhesive parameters established in this study, which aimed to determine the chemical modification of dental hard tissues and bonding stability, it can be concluded that enamel and dentin alterations depend on the radiation dose, which influence the bond strength over time. Low radiation doses (20 Gy) generated partial changes in the organic phase and decreased the microhardness of both dental hard tissues; this dose of radiation did not have a significant impact on the behavior of the bond strength of the adhesive strategies. In both time frames, doses of 40 Gy represent an inflection point affecting the adhesive performance and begin to decrease the bond strength, due to the deleterious chemical change parameters of enamel and dentin, mainly in self-etching. Although the etch-and-rinse strategy demonstrates better adhesive performance, it also generates cohesive fractures in the enamel.

These results contribute to making visible the need for consistent dental monitoring of patients, during and after radiotherapy. Such observations deliver new knowledge for clinicians: timely treatment based on adhesive strategies, allowing for more stable dental restoration over time, all dependent on the radiation dose.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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