Impact of radiotherapy on the bond strength of different adhesive systems to human dentin: one-year in vitro evaluation

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Aim: The aim of this study is to evaluate the effect of radiotherapy on the bond strength of resin-based composite restorations to dentin, performed either 24 h or 1 year before or after radiation. Methods: Ninety-six posterior teeth were randomly distributed into the following groups: IB (n = 16), irradiated teeth were restored 1 year after x-ray application; NB (n = 16), not irradiated teeth were stored for 1 year and then restored. IA (n = 32), teeth were restored and irradiated at 24 h or 1 year after the restoration. NA (n = 32), teeth were restored, not irradiated, and tested as IA. Eight samples from each group were randomly assigned to either the three-step or two-step etch-and-rinse adhesive system procedure. The irradiated specimens were subjected to 60 Gy of x-ray radiation fractionally. The restored teeth were vertically sectioned, and 1-mm² resin–dentin sticks were obtained and submitted to the microtensile bond strength test. The bond strength data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's test (p < 0.05). Failure modes were examined by optical microscopy and scanning electron microscopy. Results: The IB group showed lower bond strength values compared to the NB group. The bond strength values between the adhesive systems were not statistically different. Conclusion: The application of radiation dose decreased the bond strength of the adhesive restorations to dentin when the bonding procedure was conducted 1 year after in vitro radiotherapy.

Keywords: Dentin. Tensile strength. Radiotherapy. Head and neck neoplasms.
Introduction

Radiotherapy is the most common modality of treatment for malignant tumors in the head and neck regions. This treatment uses high-energy x-rays at doses of 40–60 Gy, and even low doses may cause changes in normal tissue located within the irradiation field, drastically diminishing the quality of life of irradiated patients. Regarding radiation-induced damage to the orofacial tissues, severe alterations in these tissues have been documented, such as mucositis, candidosis, hyposalivation, radiation caries, dysgeusia, trismus, and osteoradionecrosis. Radiation caries is one of the principal consequences of radiotherapy that results in severe destruction of human dentition.

According to some studies, the development of radiation caries is related to indirect or direct radiogenic damage to dental hard tissues. The indirect effects include salivary changes, hyposalivation, changes in the oral microbiota, limitations in performing adequate oral hygiene, and adoption of a soft diet due to swallowing difficulties. With respect to the direct effects, there is a direct alteration in biological molecules, which appear to have a negative effect on the dentinoenamel junction, enamel, dentin, and pulp components of teeth, increasing the severity of dentition breakdown. Morphological alterations of the dentin structure such as degeneration of the odontoblast processes and obliteration of the dentin tubules also have been observed. In addition, previous studies have indicated the presence of x-rays-induced damage to collagen present in the main peptide chains of dentin.

The protocol for dental restorations in oral cancer patients is still under controversial discussion. Hence, the choice of the best restorative material for dental restorations in patients undergoing radiotherapy seems to be based on the clinical experience of the professional. Recent studies have recommended the use of adhesive restorative techniques for the treatment of irradiated patients. In these studies, irradiation treatment did not affect the bond strength of the adhesive restorations to the dentin and enamel structure, when the restoration was carried out before radiotherapy. On the other hand, when the restoration protocol was performed after the application of radiation, lower bond strength values for irradiated teeth were observed compared to teeth that had not been irradiated. However, there is limited information regarding the direct effects of x-rays on the bond strength of resin-based composite restorations to enamel and dentin as well as whether the restorative procedure should be carried out before or after irradiation.

As we wanted to develop a restorative protocol based on adhesive materials for irradiated patients and to determine whether the restorative procedure should be carried out before or after irradiation, the aim of this study was to evaluate the effect of radiotherapy on the bond strength of resin-based composite restorations to dentin, using a three-step etch-and-rinse and a two-step etch-and-rinse adhesive, performed either 24 h or 1 year before or 1 year after the radiation treatment. The null hypotheses tested in this study were as follows: 1) The bond strength of the resin-based composite restorations to dentin would not be affected by radiotherapy either before or after restoration placement. 2) There would be no difference in the dentin bond strength...
between the three-step etch-and-rinse and two-step etch-and-rinse adhesive systems for all study conditions.

**Materials and methods**

Ninety-six intact, non-caries, unrestored posterior human maxillary/mandibular molars extracted over the course of three months, were obtained under a protocol approved (0394/11) by the institutional review board of Federal University of Paraiba, Brazil. The teeth were stored in 0.2% thymol solution.

**Sample preparation**

All tooth roots were embedded using self-curing acrylic resin. The teeth were randomly distributed into the following groups: IB (n = 16), irradiated teeth were restored 1 year after x-ray application; NB (n = 16), not irradiated teeth were stored for 1 year and then restored; IA (n = 32), teeth were restored and irradiated at 24 h or 1 year after the restoration. NA (n = 32), teeth were restored, not irradiated, and tested as IA. Eight teeth from each subgroup were randomly assigned to one of two adhesive system protocols: three-step etch-and-rinse adhesive (Adper™Scotchbond MP Plus, 3M/ESPE, St. Paul, MN, USA - SC) and two-step etch-and-rinse adhesive (Adper™ Single Bond 2, 3M/ESPE, St. Paul, MN - SB). Compositions and batch numbers of each material are shown in Table 1.

**Table 1. Composition and batch numbers of materials used.**

<table>
<thead>
<tr>
<th>Product (Batch number)</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Manufacture's instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adper™Scotchbond MP Plus Lot. N133000</td>
<td>3M/ESPE, St. Paul, MN, USA</td>
<td>Primer: water, HEMA. Adhesive: Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, photoinitiator.</td>
<td>Apply the phosphoric acid 37 % for 15 s, following rinse for 30 s. Apply 1 coat of primer and gently air dry for 5 s. Apply two consecutive coats of adhesive and applying a gentle stream of air for 10 s. Light-cured for 10 s.</td>
</tr>
<tr>
<td>Adper™ Single Bond 2 Lot. N 30077</td>
<td>3M/ESPE, St. Paul, MN, USA</td>
<td>Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, photoinitiator, water, ethanol</td>
<td>Apply the phosphoric acid 37 % for 15 s, following rinse for 30 s. After apply two consecutive coats of adhesive and applying a gentle stream of air for 10 s. Light-cured for 10 s.</td>
</tr>
<tr>
<td>37% Phosphoric acid etching Gel</td>
<td>FGM, Joinville, SC, BR</td>
<td>Cobalt Aluminate Blue Spinel</td>
<td>Apply the phosphoric acid 37 % for 15 s, following rinse for 30 s.</td>
</tr>
<tr>
<td>Optilux Plus</td>
<td>GNATUS, Ribeirão Preto, SP, BR</td>
<td>LED light cure</td>
<td>Light-cured using a irradiance &gt;400 mW/cm²</td>
</tr>
</tbody>
</table>

For restorative treatment after or before the application of x-rays, the occlusal enamel surface was removed using a diamond disc mounted in a low-speed laboratory cutting machine (Labcut 1010, Extec, Enfield, CT, USA) under cooling conditions. The superficial dentin was exposed and finished with 600-grit silicon carbide abrasive paper for 60 s in polishing machine (Politriz ERIOS – 27000, São Paulo, SP, Brazil),
and a flat dentin surface was obtained. The adhesives were applied according to the manufacturers’ instructions (Table 1). After the adhesion process, the resin block was built up using three layers of Z350 composite resin (3M/ESPE, St. Paul, MN, USA), resulting in a height of 4.5 mm. Each layer was light cured for 40 s with an intensity of 400 mW/cm² (Optilux Plus GNATUS, Ribeirão Preto, São Paulo, Brazil).

Radiotherapy
The simulated radiotherapy was performed using a Primus K Linear Accelerator (Siemens Healthineers, USA) with an energy of 6 MeV, a source–surface distance of 100 cm, and a field size of 18 cm × 23 cm. The specimens were placed in individual containers, which were filled with distilled water above the resin blocks, in order to provide dose homogeneity. Radiation was applied perpendicular to the surface of the specimen, and a total dose of 60 Gy, in fractions, was delivered. All groups of specimens were stored in distilled water, changed daily, at 37 °C.

Microtensile bond strength test
For the bond strength test, the restored teeth were sectioned longitudinally in the mesio-distal and buccal-lingual directions across the bonded interface, using a slow-speed diamond saw (Isomet, 1000 Buehler Ltd., Lake Bluff, IL, USA) to obtain 15–30 resin–dentin sticks with a cross-sectional area of approximately 1 mm² (±0.1 mm²). The resin–dentin bonded sticks were fixed to a testing jig with cyanoacrylate glue (Super Bond Gel, Loctite Brazil Ltd.) and subjected to the tensile load at a crosshead speed of 0.5 mm/min until failure (Shimatzu, Kyoto, Japan). The microtensile bond strength was expressed in MPa and derived by dividing the imposed force (N) at the time of fracture by the bond area (mm²).

Failure mode analysis
The fractured surfaces of all specimens were observed by using an optical microscope (XJM-400, KOZO, Nanjing, China) at a magnification of 100×. The fracture mode was classified as follows: (I) cohesive failure in the adhesive, (II) cohesive failure in the dentin, (III) cohesive failure in the hybrid layer, or (IV) mixed failure, cohesive failure in the adhesive and cohesive failure in the hybrid layer. Representative fractured surfaces of each tested group exhibiting the most frequently observed failure mode were analyzed by scanning electron microscopy (JSM-5600, JEOL, Tokyo, Japan) operating at 15 kV and a working distance of 15 mm.

Statistical analysis
Bond strength data were submitted to two-way (adhesive system vs. moment of radiation) analysis of variance (ANOVA) followed by Tukey’s test at a significance level of p < 0.05.

RESULTS
ANOVA revealed that the material, radiation, and all possible interactions between the factors resulted in statistically significant differences (p < 0.005) for the two storage times (24 h and 1 year). There was no statistical difference in the bond strength values
between the control group (not irradiated) and irradiated group restored before radiotherapy, using both adhesive systems, after short- and long-term storage (24 h and 1 year) (Tables 2 and 3). When the adhesive restorations were carried out one year after radiation application, the irradiated group presented lower bond strength values compared to the control group (Table 4). There was no statistical difference in the bond strength values between the adhesive systems used for all study conditions. No pretesting failures were recorded for any group.

Table 2. Means values of bond strength (MPa) and standard deviation of adhesive restorations performed before the radiation in 24 hours storage.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adhesive system</th>
<th>Single Bond</th>
<th>Scotch Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
<td>Single Bond</td>
<td>39,64 ± 4,9</td>
<td>Aa</td>
</tr>
<tr>
<td>Control (no irradiated)</td>
<td>Single Bond</td>
<td>41,67 ± 7,6</td>
<td>Aa</td>
</tr>
<tr>
<td></td>
<td>Scotch Bond</td>
<td>38,28 ± 9,9</td>
<td>Aa</td>
</tr>
<tr>
<td></td>
<td>Scotch Bond</td>
<td>41,43 ± 10,06</td>
<td>Aa</td>
</tr>
</tbody>
</table>

Groups identified with different upper case letter superscripts (analysis in rows) and lower case letters (analysis in columns) represent statistical significant differences (p<0.05).

Table 3. Means values of bond strength (MPa) and standard deviation of adhesive restorations performed before the radiation in 1 year storage.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adhesive system</th>
<th>Single Bond</th>
<th>Scotch Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
<td>Single Bond</td>
<td>39,65 ± 5,44</td>
<td>Aa</td>
</tr>
<tr>
<td>Control (no irradiate)</td>
<td>Single Bond</td>
<td>40,67 ± 5,67</td>
<td>Aa</td>
</tr>
<tr>
<td></td>
<td>Scotch Bond</td>
<td>38,15 ± 9,19</td>
<td>Aa</td>
</tr>
<tr>
<td></td>
<td>Scotch Bond</td>
<td>40,34 ± 7,90</td>
<td>Aa</td>
</tr>
</tbody>
</table>

Groups identified with different upper case letter superscripts (analysis in rows) and lower case letters (analysis in columns) represent statistical significant differences (p<0.05).

Table 4. Means values of bond strength (MPa) and standard deviation of adhesive restorations performed after the radiation in 1 year storage.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adhesive systems</th>
<th>Single Bond</th>
<th>Scotch Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
<td>Single Bond</td>
<td>35,39 ± 7,47</td>
<td>Ab</td>
</tr>
<tr>
<td>Control (no irradiated)</td>
<td>Single Bond</td>
<td>41,33 ± 5,09</td>
<td>Aa</td>
</tr>
<tr>
<td></td>
<td>Scotch Bond</td>
<td>32,68 ± 7,45</td>
<td>Ab</td>
</tr>
<tr>
<td></td>
<td>Scotch Bond</td>
<td>41,01 ± 7,63</td>
<td>Aa</td>
</tr>
</tbody>
</table>

Groups identified with different upper case letter superscripts (analysis in rows) and lower case letters (analysis in columns) represent statistically significant differences (p<0.05).

Table 5 and 6 show the mode of failure. The predominance of cohesive failure in the adhesive was detected for the control and irradiated groups restored 24 h and 1 year before radiotherapy. On the other hand, mixed failures (cohesive failure in the adhesive and cohesive failure in the hybrid layer) predominated when the specimens were restored 1 year after radiotherapy (Fig. 3).
DISCUSSION

According to the results of this study, the bond strength of the adhesive systems tested to dentin decreased when the restorations were performed 1 year after radiation application. Moreover, no difference in the dentin bond strength between those adhesive systems was observed for all study conditions. Consequently, both hypotheses of this study were rejected. Therefore, the restorative treatment for oral cancer patients who have dental cavities should be carried out before initiating head and neck radiotherapy, because irradiation doses may modify human tissues structure, as enamel and dentin, impairing the formation of an adequate bond between adhesive treatment and tooth structure.

The ionizing radiation used as a treatment for oral cancer patients has a short wavelength and a high energy, which may induce micro-morphological alterations in dentin and enamel. The results of this study showed that radiotherapy caused a
significant detrimental effect on the bond strength of adhesive systems to dentin when the adhesive restorations were carried out 1 year after radiation application to the teeth, using two- or three-step adhesives. This fact may be explained by a direct effect of high-dose radiation (60 Gy) on the dentin structure. It is well known that radiation reacts with water, forming hydrogen and hydrogen peroxide free radicals\(^2\). Dentin contains a considerable amount of water in its composition; therefore, x-rays act through the formation of free radicals, which may have a negative effect on the secondary and tertiary structures of dentin proteins, causing the loss of collagen fiber hydration and leaving the tissue dry and friable\(^7,24\). As a result of this process, some micro-morphological alterations in the dentin structure can occur, including collagen fiber fragmentation\(^7,15\) and obliteration of dentin tubules, which is preceded by degeneration of odontoblast processes\(^14\). This damage may impair formation of the hybrid layer, producing a permeable adhesive interface between the adhesive system and the irradiated dentin. Moreover, alteration of the structural organization of collagen may occur because some chemical bonds are broken by free radicals during radiation and reorganization of the chemical components may happen, thus altering the structure\(^15\). In this study, a self-adhesive system was not assessed and further investigation is required to evaluate if that adhesive system will produce a strong and durable adhesive interface with the irradiated dentin, since self-adhesive systems containing an acidic monomer, as MDP, is capable to bond chemically to dentin and enamel structure\(^25\).

The results of this in vitro study corroborate with previous findings, which showed the lowest bond strength values to dentin when the adhesive restoration was carried out after radiotherapy\(^19,21,26\). However, in these studies, the adhesive procedure was performed immediately or 24 h after radiation therapy. In clinical conditions, dental bonding procedures are not performed immediately after finishing radiotherapy. Thus, in our study, the restorative procedure was carried out 1 year after the application of radiation to the teeth. This is the first study to evaluate the long-lasting effect of radiation on the dentin structure and its consequences on the bonding effectiveness of adhesive systems to irradiated dentin. A previous report has hypothesized that in vivo high-dose radiotherapy causes induction and activation of enzymes that degrade collagens over a period of months or years\(^27\). The effect of radiation (60 Gy) on the dentin microstructure could be observed by the mode of failure for the irradiated group restored after radiotherapy (Table 6). The predominant fracture modes were mixed failures (cohesive failure in the adhesive and cohesive failure in the hybrid layer), which showed failure of the formation of a stable and strong hybrid layer (Figure 1).

Regarding the group in which the restorative procedure was carried out before the radiation application, there were no significant differences for the bond strength values between the irradiated and nonirradiated restored teeth after storage for 24 h or 1 year. This finding is in accordance with others study\(^6,19,28\) which allege that when hybridization is obtained prior to irradiation, the alterations in the substrate might not be great enough to affect the behavior of the pre-existing hybrid layer and to compromise the bonding effectiveness between dentin and the adhesive materials. For all groups in which the teeth were restored before radiation, the predominant failure mode was cohesive failure in the adhesive (Table 5). It is possible that radiation applied after
the restorative procedure did not influence the bond quality of the adhesive material to dentin; consequently, adequate bond strength values could be obtained.

As observed in the present investigation, the application of x-rays had a harmful effect on the bond strength to human dentin when the adhesive restorations were placed 1 year after radiotherapy. Nevertheless, this scenario could be different in clinical practice considering the dry mouth of patients, increased viscosity, decreased salivary pH, dietary changes, and deficiencies in oral hygiene during and after radiotherapy. Therefore, future research should be conducted to simulate intraoral conditions in order to validate the findings of this study.

Within the limitations of the current study, it was concluded that the application of x-rays decreased the bond strength of the tested adhesive restorations to dentin when the bonding procedure was conducted 1 year after in vitro radiotherapy. In addition, the different adhesive systems used in this study (two- or three-step etch-and-rinse procedure) showed similar bond effectiveness to dentin, regardless of the time period after the restoration was introduced.

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REFERENCES


