Radiotherapy Impairs Adhesive Bonding in Primary Teeth: An In Vitro Study

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ABSTRACT

Purpose: To evaluate the morphological alterations in enamel and dentin of primary teeth following radiotherapy (RT) and to determine the best adhesive technique and time to carry out restorative procedures.
Methods: Enamel and dentin fragments of primary teeth were randomly assigned into four groups (N=30): G1 (control)—non-irradiated, only restorative procedure; G2—restorative procedure immediately before RT; G3—restorative procedure 24 hours after RT; and G4—restorative procedure six months after RT. Each group was divided into one of two subgroups according to the adhesive system used for restoration: (1) AdperSingle Bond 2 (SB); and (2) ClearfillSE Bond (CL). The specimens were submitted to fractionated RT until they reached the final dose of 60 Gy. They were then subjected to confocal microscopy and the shear bond strength test. Data were analyzed using two-way analysis of variance followed by Tukey's tests (α= five percent).
Results: Morphological changes were first observed in enamel and dentin after 40 Gy of irradiation. G4 bond strength values were similar to G1 in the CL and SB groups for enamel and in the CL group for dentin (P>0.05). G2 showed the lowest values for enamel and dentin (P<0.05). In G3, CL presented the highest strength values in enamel; for G4, the highest values were found in dentin (P<0.05).
Conclusions: Radiotherapy affected the morphological surface of enamel and dentin. The restorations placed immediately after RT had the weakest shear bond strength, and the restorations placed six months after RT had similar means of bond strength compared to the nonirradiated teeth in enamel, regardless of the adhesive system used. In dentin, CL showed better performance than SB. **(J Dent Child 2020;87(2):69-76)**
Received February 25, 2019; Last Revision December 10, 2019; Accepted December 10, 2019.

KEYWORDS: RADIOTHERAPY, HEAD AND NECK CANCER, DENTAL BONDING, PRIMARY TEETH, RADIATION CARIES, ADHESION, SHEAR BOND STRENGTH, MORPHOLOGY

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Head and neck cancer (**HNC**) is predominately represented by squamous cell carcinomas of the upper aerodigestive tract. In children, HCN comprises rhabdomyosarcomas,[1] nasopharyngeal carcinoma,[2,3] epipharyngeal carcinoma,[3] thyroid carcinoma,[4] head and neck bone sarcoma,[5] nuclear protein in testis (NUT) carcinoma,[6] and medulloblastoma.[7] Pediatric HCN represents 12 percent of all cancers.[8] Radiotherapy (**RT**) is a therapeutic modality used as a main or adjuvant treatment for these cases.[1-6] RT with chemotherapy concurrent may be used in primary treatment for unresected cancers of the head and neck, or postoperative radiotherapy (**PORT**) may be indicated for positive resection margins, extracapsular spread in lymph node metastases, and other minor factors.[9]

Despite the high rates of success with RT, there are many side effects, including mucositis, xerostomia, taste loss, trismus, progressive loss of the periodontal ligament, soft tissue necrosis, osteoradionecrosis, and radiation-related caries.[10] Long-term structural alterations in enamel and dentin due to RT are also observed in primary and permanent teeth.[11-17] To reduce RT toxicity, conventional fractionation protocols for HNC are defined by once-daily treatment fractions of two Gy at a total dose, with treatment techniques including computerized three-dimensional RT treatment planning or intensity-modulated radiotherapy. The doses per fractionation radiotherapy used are 66 to 70 Gy per 33 to 35 daily fractions in a primary tumor and any involved lymph nodes; 60 to 66 Gy per 30 to 33 daily fractions in PORT; and 50 to 54 Gy per 25 to 27 daily fractions in regions to be treated to an elective dose. Severe late toxicity after HNC treatment is common, affecting approximately half of patients. Older age, advanced T-stage, and larynx/hypopharynx as a primary site were strong independent risk factors.[18,19]

Prior to RT, it is necessary to remove or at least reduce infectious foci in the oral cavity, which includes treating dental caries. After RT to treat HCN, radiation-related caries is a very common side effect,[10,20,21] which should be treated via tooth restoration. Conventional glass ionomer cements are not the first option for restorative procedures,[22] as xerostomia is one of the main side effects of RT and affects largely glass ionomer cement, causing erosion of the material.[13,16,23,24] For these reasons, composite resins, using etch-and-rinse or self-etch adhesive systems for bonding, are indicated as restorative materials.[25,26] However, it has been reported that the bonding strength of adhesives could be affected by both RT and RT-related oral side effects.[27] In permanent teeth, the performance of adhesive systems in irradiated teeth is contradictory;[17,28-31] no studies have evaluated the shear bond strength (**SBS**) of resin systems in primary teeth undergoing RT.

The cure rates for cancer patients have increased due to medical advances resulting in early diagnosis and more precise treatments.[32,33] The rates of long-term survivors with a high risk of medulloblastoma are up to 70 percent,[7] increasing the probability that pediatric survivors will need dental treatment. Therefore, pediatric dentists must be able to treat these patients in order to perform the best clinical treatment based on scientific evidence. Because it was previously found that morphological alterations and decrease of microhardness occur after RT in primary teeth,[11] we hypothesized that those changes would affect the bonding strength of adhesives.

The aim of this in vitro study was to determine the best adhesive technique (between etch-and-rinse and self-etch) and the best time (pre- or post-radiotherapy) to carry out restorative procedures in primary teeth submitted to RT, according to the adhesive shear bonding strength.

METHODS

ETHICAL ASPECTS AND SAMPLE

This study was approved by the Ethics Committee at the School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. Sixty healthy, freshly extracted human primary maxillary and mandibular second molars were obtained and stored in distilled water at four degrees Celsius.

RADIOTHERAPY PROCESS

The teeth were submitted to a fractionated dose of two Gy over five consecutive days for six weeks, with a final dose of 60 Gy.[11,12,34-36] The radiation was emitted from an irradiator (RS 2000; Rad Source Technologies, Suwanee, Ga., USA), using the energy of 200 kVp and 25 mA and a default copper filter measuring 0.3 mm. Radiation generated under this condition has a spectrum with minimum and maximum energy values of 95 and 200 kV, respectively, and half the value of the beam with 0.62 mm of copper. The gradient of this radiation dose in tissues is approximately 10 percent at a depth of 0.5 cm. Plates were aligned equidistant from the center of the beam and inside the cone to ensure a uniform dose rate (approximately 2.85 Gy per minute) and total delivery of the fractionated dose. During and after RT, the specimens were kept in artificial saliva at 37 degrees Celsius (artificial saliva contained [g/L]: methyl-*p*-hydroxybenzoate 2.00; sodium carboxymethyl cellulose 10.0; KCL 0.625; $MgCl_2.6H_2O$ 0.059; $CaCl_2.2H_2O$ 0.166; K_2HPO_4, 0.804; and KH_2PO_4 0.326, according to Amaechi et al.[37]); the pH was adjusted to seven using potassium hydroxide (KOH).

GROUP ASSIGNMENT

Roots were removed approximately one mm below the cementoenamel junction using a refrigerated cutting machine (Miniton, Struers A/S, Copenhagen, Denmark). The crown was cut in the mesiodistal direction and then in the buccal-lingual direction, resulting in four fragments per tooth, for a total of 240 fragments. The fragments were divided into enamel (N=120) and dentin (N=120). The specimens were randomly assigned into four groups, according to time of restoration of both substrates (i.e., enamel and dentin (N=30 per group): (1) G1 (control)—non-irradiated, only restorative procedure; (2) G2—restorative procedure prior to RT; (3) G3—restorative procedure immediately after RT; and (4) G4—restorative procedure six months after RT. After the group assignment, each group was divided into two subgroups according to the adhesive system used: (1) restorations done using AdperSingle Bond 2 (**SB**; 3M/ESPE, St. Paul, Minn., USA); and (2) restorations done using ClearfillSE Bond (**CL**; Kuraray Co. Ltd., Umeda, Osaka, Japan).

SPECIMEN PREPARATION AND RESTORATIVE PROCEDURE

The fragments were cleaned, included in acrylic resin, and polished in a DP-9U2 polishing machine (Strues A/S, Panambra, Copenhagen, Denmark) to obtain a plane surface, and to standardize the smear layer. The specimens were washed in running water, dried with gauze, and placed in ultrasonic cleaner using water for five minutes to remove possible debris. To carry out the restoration process, a Teflon matrix was used to standardize the restorations, resulting in a resin cylinder measuring four-mm high and two mm in diameter bonded to the dental surface.

The adhesive systems were applied to the bonding areas according to the sample subgroups and manufacturer's instructions. The chosen composite resin was Z350 (3M Dental Products, St. Paul), which was inserted in increments and polymerized with 1,000 mW/cm^2 of power (DB 686, Dabi Atlante, Ribeirão Preto, Brazil).

MORPHOLOGICAL ANALYSIS BY LASER CONFOCAL MICROSCOPY

For the morphological analyses, 10 specimens (five of enamel and five of dentin substrate) were randomly selected and analyzed using a laser confocal microscope (LEXT OLS4000, Olympus, Waltham, Mass., USA). These analyses were done before RT and after every 10 Gy of exposure to radiation until reaching the final dose of 60 Gy. The analyzed area of each specimen was standardized at the microscopy to perform all analyses in the same area.

SBS TEST

Twenty-four hours after the final dose of radiation, the samples were subjected to SBS measurement in a blinded manner using a knife-edge blade in a universal testing machine (model 2519-106, Instron, Canton, Mass., USA) with a vertical speed of 0.5 mm per minute using a load cell of 20 kgf, and the values were measured (N) and converted to MPa.

FRACTURE PATTERN ANALYSIS BY LASER CONFOCAL MICROSCOPY

The surface of all specimens was analyzed using the laser confocal microscope to evaluate the fracture pattern after the shear bond test. Each specimen was classified according to the predominant remaining structure: adhesive fracture (between the bonding agent and the surface); cohesive fracture within dental substrate (either enamel or dentin); cohesive fracture within bonding agent and/or composite resin; and mixed fracture involving a bonding agent and/or composite resin and/or tooth structure (at least 40 percent of the surface of each type of fracture). The confocal analysis was conducted by three blinded trained and calibrated researchers (kappa greater than 0.8).

STATISTICAL ANALYSIS

Data of the SBS test (in MPa) were analyzed for normality and homogeneity using Shapiro-Wilk and Levene tests, respectively. Parametric analysis by means of two-way analysis of variance (time of restoration and bonding agent) was used and complemented via Tukey's test. Data were analyzed using Bioestat 5.3 (Instituto Mamirauá, Tefé, Amazonas, Brazil) at a significance level of five percent.

RESULTS

MORPHOLOGICAL SURFACE

During and after RT, enamel and dentin presented morphological changes compared to non-irradiated primary teeth. In enamel, slight alterations at the surface

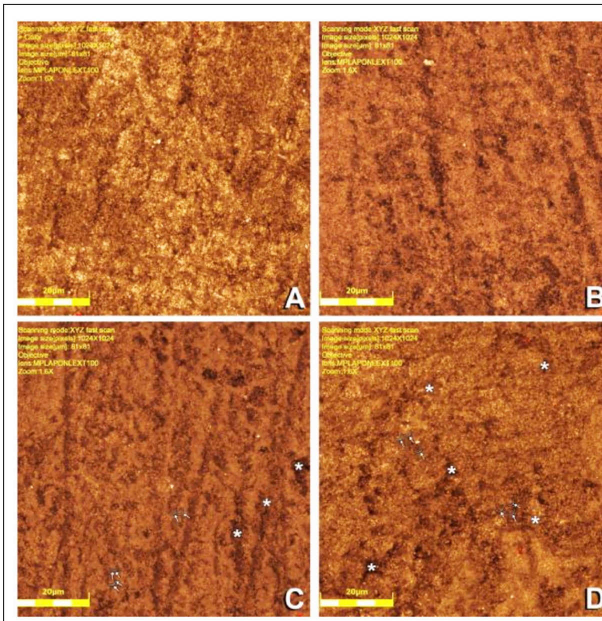

Figure 1. Representative confocal microscopy images of primary enamel (3,415 x magnification): (A) normal structure, before radiotherapy; (B) slight alterations at 20 Gy; (C) more evident alterations at 40 Gy; and (D) flattered aspect of the surface at 60 Gy.

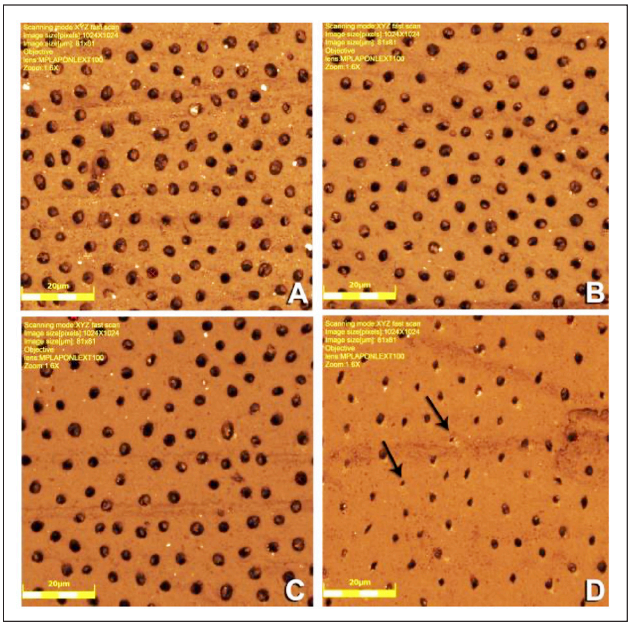

Figure 2. Representative confocal microscopy images of primary dentin (3,415x magnification): (A) normal open tubules before radiotherapy; (B) slight reduction of the tubule's diameter at 20 Gy; (C) fewer open tubules at 40 Gy; and (D) at 60 Gy, most of the tubules were obliterated.

Table 1. Shear Bond Strength (MPa) Mean of Adhesive Systems in Enamel*		
Group	AdperSingle Bond 2	Clearfill SE Bond
G1	20.34 a A	19.29 b A
G2	7.78 b A	8.64 a A
G3	10.65 b B	25.41 b A
G4	24.93 a A	25.69 b A

* *Lowercase letters indicate comparison of the same adhesive among the different periods of restoration; those with the same lowercase letters are not significantly different (P>0.05). Capital letters indicate a comparison of the different adhesive in the same period of restoration; those with the same capital letters are not significantly different (P>0.05).*

Table 2. Shear Bond Strength (MPa) Mean of Adhesive Systems in Dentin		
Group	AdperSingle Bond 2	Clearfill SE Bond
G1	26.56 a A	18.08 a B
G2	7.80 b A	5.40 b A
G3	10.79 b A	14.61 a A
G4	13.52 b A	21.70 a B

* *Lowercase letters indicate comparison of the same adhesive among the different periods of restoration; those with the same lowercase letters are not significantly different (P>0.05). Capital letters indicate a comparison of the different adhesive in the same period of restoration; those with the same capital letters are not significantly different (P>0.05).*

were observed after 20 Gy, which became more evident after 40 Gy. At 60 Gy, a flattered pattern was clearly observed (Figure 1). For dentin, after 20 Gy, there was a slight reduction in the diameter of the tubules. At 40 Gy, fewer open tubules were observed in comparison to 20 Gy irradiation. At 60 Gy, most tubules had a significant reduction in their diameter (Figure 2).

SBS TEST

In enamel, using the SB adhesive system, the SBS in restorations placed six months after RT (G4) was not different from restorations placed in non-irradiated teeth (G1; $P>0.05$). On the other hand, SBS was significantly reduced in restorations performed prior to RT (G2) or immediately after RT (G3; $P<0.05$). Using the CL adhesive system, the SBS in restorations performed immediately following RT (G3) or six months after RT (G4) or in nonirradiated teeth was similar ($P>0.05$) but significantly higher than that found in restorations performed prior to RT (G2; $P<0.05$; Table 1).

In dentin, using the SB adhesive system, the SBS in restorations performed prior to RT (G2), immediately following RT (G3), or six months after RT (G4) was significantly reduced compared to restorations performed in non-irradiated teeth (G1; $P<0.05$). Using CL adhesive system, the SBS in restorations performed immediately

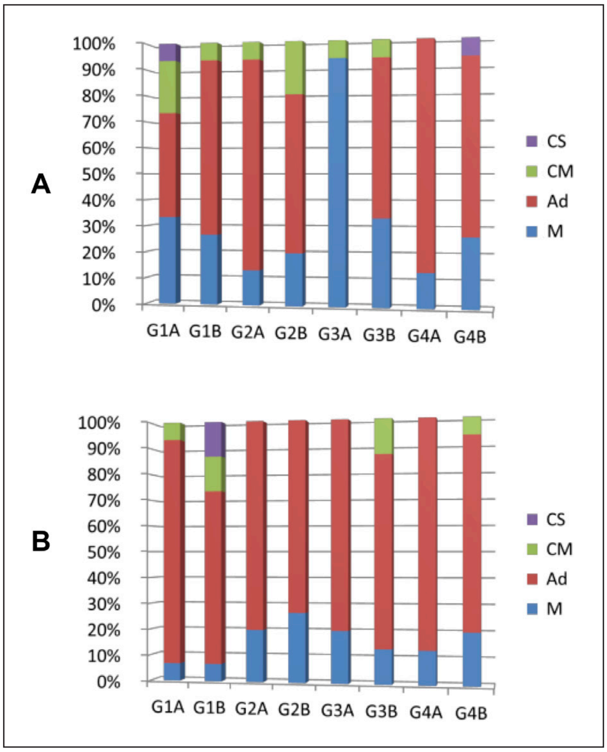

Figure 3. Percentage of fracture patterns of restorations in primary tooth restorations performed in nonirradiated teeth (group one) and in restorations performed prior to radiation (group two), immediately following radiation (group three), or six months after radiotherapy (group four). CS=cohesive substrate fracture pattern; CM=cohesive material fracture pattern; Ad=adhesive fracture pattern; M=mixed fracture pattern; CL=Clearfill SE Bond; SB=AdperSingle Bond 2. (A) Enamel. (B) Dentin.

following (G3) or six months after RT (G4) was similar to non-irradiated teeth ($P>0.05$). On the other hand, the SBS was significantly lower in restorations performed prior to RT (G2; $P<0.05$; Table 2).

FRACTURE PATTERN

Analyzing the percentage of the fracture pattern in enamel and dentin, an adhesive fracture was predominant in most of the groups. Of note, in restorations performed immediately after RT (G3), there was a high percentage of mixed fractures (95 percent for SB and 32 percent for CL) in enamel. In dentin, for restorations in non-irradiated teeth (G1), CL was the only subgroup that had a presence of cohesive fracture (12 percent) within the dental substrate (Figure 3).

DISCUSSION

As a common side effect, disintegration of dental tissues after RT happens frequently due to direct effects on tooth structure[11,12] and indirect effects mostly due to saliva diminution.[13,16] This disintegration process has peculiar characteristics, such as fast progression and predilection to the cervical portion of the tooth.[10,21] Disintegration is usually named radiation-related caries, and composite resins are widely used for restorations in both permanent and primary teeth.[25]

The RT protocol for HNC used in this study was followed by recent studies[17,35,36] and consisted of cumulative fractionated doses of two Gy and daily sessions during weekdays until the final dose of 60 Gy.[10,12,17,35,36] The fractionated RT protocol was followed to respect the 5Rs (repair, redistribution, reoxygenation, regeneration, and radiosensitivity).[38]

The observed morphological changes on the surface of primary teeth by confocal microscopy agree with the findings by SEM previously presented in primary teeth[11] and also with those found in permanent teeth.[12,39] The most evident alterations occurred in dentin after 40 Gy, with progressive obliteration of dentin tubules until reaching the final dose of 60 Gy. We have demonstrated that the same RT protocol used in this study caused morphological alterations in permanent teeth.[17] However, more studies in primary teeth are necessary since there are substantial differences in the microstructure of primary versus permanent enamel and dentin. Among those differences, a lower level of calcium and phosphorus and lesser thickness can be highlighted.[40] In dentin there are microchannels or giant dentin tubules; therefore, the area of solid dentin that is available for dentin bonding is significantly reduced.[41]

According to our results, the SBS was negatively affected by RT; G2 presented the worst results, regardless of the adhesive system applied. However, the SBS was not affected in G3 and G4 using the CL system in enamel and dentin. This result agrees with Keles et al.,[42] even though the studied materials were different. They evaluated the microtensile bond strength of polyacid-modified composite resins (compomer) and observed that RT does not affect the microtensile bond strength in primary teeth enamel.

The SB system was more affected by RT; the SBS was negatively affected in enamel and dentin when restorations were carried out immediately after RT and in dentin six months after RT. Impaired adhesion was also observed by Keles et al.[42] after compomer restorations in dentin were submitted to RT. The authors hypothesized that the restoration-tooth interface is negatively affected by RT due to the significant morphologic alterations in dentin, such as more evident interprismatic portion, presence of obliterated dentinal tubules and fissures, fragmentation of the collagen fibers, and degradation of peritubular dentin.

After RT, significant changes in the mineral and organic components of enamel and dentin occur, especially alterations in phosphate, carbonate, amide, and hydrocarbons in enamel and alterations in phosphate, amide, and hydrocarbon in dentin.[15] It is possible that these alterations might influence adhesion of the composite resins on dental substrates, since the adhesion process depends on organic and inorganic components. Additionally, RT increased gelatinase (MMP-2 and -9) activity in all regions of the dentino-enamel junction in permanent teeth, which could enhance degradation of the hybrid layer and compromise adhesion to the dental substrate.[43]

To the best of our knowledge, this is the first study that has evaluated the SBS of composite restorations after RT in primary teeth. Only a few studies have evaluated the effects of RT on different times of restorative procedures in permanent teeth. We found that RT substantially changes the morphological surface of enamel and dentin and impairs the bonding strength. The CL system had better results than the SB system, and restoring teeth before RT showed the worst results for enamel and dentin.[17]

The best restorative materials for dental treatment after RT are composite resins, which use adhesive systems for bonding.[25] Composite resins have shown a longer survival rate when compared to glass ionomer cement, for example.[26] There are two different bonding systems for composite resins: etch-and-rinse and self-etch. The self-etch system has been preferred by dentists because it does not require a separate etching step, resulting in a shorter application time; it is also less technique sensitive, making its clinical performance more reliable.[44-46] Another clinically relevant aspect of the self-etch system is the lower incidence of postoperative sensitivity compared to the etch-and-rinse system,[47-49] which might be explained by the less aggressive and more superficial interaction with dentin when compared to the phosphoric acid used in the etch-and-rinse system.[50]

The CL system, which is a self-etch adhesive system, presented better results in this study, which corroborates those of a systematic review suggesting that self-etch adhesives should be used to restore the teeth of head and neck cancer patients either before or after radiation.[27] The acid used in etch-and-rinse adhesives promotes microporosities to improve the penetration of the bonding agent,[51] and, as previously demonstrated, the enamel becomes more porous after radiotherapy.[11,12] The use of acid on the impaired enamel after radiotherapy might cause an overconditioning; this might explain the best results for the SB group, since this adhesive system does not need the use of an acid.

In dentin, it is plausible that the self-etch system showed a better performance since it only partially demineralizes the substrate and also incorporates itself to the smear layer. The hydrophilic acids in this adhesive simultaneously demineralize and penetrate the dentin, avoiding the partial penetration of the adhesive in previously demineralized areas.[52] Another possible explanation is that the primers used with the self-etch system have a pH of 2.0, causing a partial demineralization of dentin and forming a uniform hybrid layer with hydroxyapatite of 0.5 to one μm, which could protect the collagen network.[44,53] Meanwhile, the etch-and-rinse systems completely demineralize the dentin, exposing the collagen, and crystals of hydroxyapatite become unprotected. The lack of studies in irradiated primary teeth makes direct comparison impossible.

The predominant fracture pattern in most of the primary teeth enamel and dentin groups was adhesive fracture (between the bonding agent and the surface), as reported previously in permanent teeth.[17,29,31] The predominance of the adhesive fracture might be due to the fact that RT negatively affected the bonding interface of dentin and the adhesive system; a hypothesis for this result might be alterations on the morphological surface of the primary dentin, especially the obliteration of the dentin tubules after RT, which was observed in the present study and that of de Siqueira Mellara et al.[11] Microhardness alterations on enamel and dentin of primary teeth after RT were previously observed as well.[11,15] These alterations could increase the instability of the substrate, leading to adhesive fractures.[34]

The good results observed in the group restored six months after RT, both in enamel and dentin, might be explained by the fact that the teeth were stored in artificial saliva at 37 degrees Celsius until the time of restoration in order to simulate the oral cavity conditions. Since the artificial saliva is rich in ions, it might allow remineralization of the teeth.

Limitations inherent to an in vitro study, such as patient biology and oral hygiene, could not be applied in this study; furthermore, the lack of studies in irradiated primary teeth makes it impossible to compare the results directly. It is recommended that patients with carious lesions in primary teeth who are recommended to receive head and neck RT have the lesions removed and provisionally restored with resin-modified glass ionomer cement before starting the radiotherapy. Here, we found that definitive restorations with composite resin using the self-etch adhesive could be placed after the RT protocol is finished or after six months, since the bond strength was similar to that for non-irradiated teeth. We believe that in vivo studies in primary teeth are necessary to confirm the results, taking into consideration the variables that were not able to be evaluated in this study.

CONCLUSIONS

Based on the results of this study, the following conclusions can be made:

1. Radiotherapy affects the morphological surface of enamel and dentin in primary teeth.
2. Restorations placed before radiotherapy have the lowest shear bond strength.
3. Restorations placed six months after radiotherapy had means of bond strength similar to non-irradiated teeth, when the self-etch adhesive was used in enamel and dentin.
4. Overall, Clearfill SE Bond presented a better performance in irradiated teeth compared to AdperSingle Bond 2.

ACKNOWLEDGMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasilia, Distrito Federal, Brazil.

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