J Oral Res
research in oral sciences

ORIGINAL ARTICLE

BOND STRENGTH OF COMPOSITE RESIN/UNIVERSAL ADHESIVE TO DENTIN SUBMITTED TO RADIOTHERAPY

Fuerza de unión de resina compuesta/adhesivo universal a dentina sometida a radioterapia

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ABSTRACT

Aim: To evaluate the bond strength of a universal adhesive system to dentin submitted to radiotherapy.

Materials and Methods: Sixty extracted human teeth were divided into two groups (n = 30): without radiotherapy (control); with radiotherapy, according to the adhesive protocol (n=15): ER-etch-and-rinse (acid + Single Bond Universal); SE-self-etch (Single Bond Universal). The analyzes were shear bond strength (SBS) (n=10), failure pattern (n=10) and scanning electron microscopy (n=5). Data was analyzed by a two-way ANOVA (α =0.05).

Results: The radiotherapy decreased SBS of the restorative material to dentin (p<0.0001). The ER protocol provided lower bond strength values (p<0.001). The predominant type of fracture without radiotherapy was mixed (SE), cohesive to the material (ER). Both protocols presented adhesive failures with radiotherapy. Teeth had a hybrid layer and long resin tags (without radiotherapy) and few tags (with radiotherapy).

Conclusions: The SE adhesive mode favors the shear bond strength of resin to dentin in teeth submitted to radiotherapy.

Keywords: *Dental Cements; Adhesives; Dental bonding; Self-curing of dental Resins; radiation treatment; Dentin.*

RESUMEN

Objetivo: Evaluar la fuerza de adhesión de un sistema adhesivo universal a la dentina sometida a radioterapia.

Materiales y Métodos: Sesenta dientes humanos extraídos se dividieron en dos grupos (n = 30): sin radioterapia (control); con radioterapia, según protocolo adhesivo (n=15): ER-grabado y enjuague (ácido + Single Bond Universal); autograbado SE (Single Bond Universal). Los análisis ejecutados fueron resistencia al cizallamiento (SBS) (n=10), patrón de falla (n=10) y microscopía electrónica de barrido (n=5). Los datos se sometieron al test de ANOVA de dos vías (α =0,05).

Resultados: La radioterapia disminuyó la SBS del material restaurador a la dentina (p<0,0001). El protocolo ER proporcionó valores de fuerza de unión más bajos (p<0,001). El tipo de fractura predominante sin radioterapia fue mixta (SE), cohesiva al material (ER). Ambos protocolos presentaron fallas adhesivas con radioterapia. Los dientes tenían una capa híbrida y colas de resina largas (sin radioterapia) o pocas colas de resina (con radioterapia).

Conclusión: El modo adhesivo SE favorece la resistencia al corte de la resina a la dentina en dientes sometidos a radioterapia.

Palabras Clave: *Cementos dentales; Adhesivos; Recubrimiento dental adhesivo; Auto-curación de resinas dentales; Radioterapia; Dentina.*

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CITE AS: de Goes Paiola F, Souza-Gabriel AE, Leite Paschoini V, Mussolino Queiroz A & Miranda Cruz-Filho A. Bond strength of composite resin/universal adhesive to dentin submitted to radiotherapy. J Oral Res. 2023; 12(1): 203-216. doi:10.17126/joralres. 2023.018

Received: January 25, 2023
Accepted: August 23, 2023
Published online: November 3, 2023

ISSN Print 0719-2460
ISSN Online 0719-2479.

INTRODUCTION

Head and neck cancer comprises malignant neoplasms present in the oral cavity, pharynx, larynx, salivary glands, nasal cavity, paranasal sinuses and thyroid.[1,2] Radiotherapy is a very common treatment modality for these cases and consists of employing high doses of radiation on the tumor.[3,4] The radiotherapy protocol should be performed in a fractionated way, minimizing side effects.[4,5] Even so, mucositis, xerostomia, loss of taste, trismus, and progressive loss of periodontal ligament, soft tissue necrosis, osteoradionecrosis and radiation-related caries are likely to arise during therapy or after it.[3,6,7] Radiation therapy has a direct and indirect impact on the dental structure, including enamel and dentin microhardness, dentinoenamel junction, and acid solubility of the enamel, and these effects were involved in the pathogenesis of the disease. [8–11]

Adhesive dental restorations are the preferred treatment for replacing lost dental structure, whether due to caries or non-carious lesions. Gaps can be found in the tooth-resin interface, and this can present bonding failures along time.[4,11,12] Failures in the interface can lead to an increase in oral biofilm accumulation and infections.

When combined with the fragility of teeth subjected to radiotherapy, these issues can result in severe consequences for patients' oral health.[4,6,11–13] The damage to the tissue structure can influence the performance of the adhesive material, and the method of application should follow the characteristics of enamel and dentin.[5,14]

In dental adhesive restorations, the primer and/or adhesive has an affinity for the exposed collagen fibrils and, on the other hand, they have hydrophobic groups, which chemically bond to the adhesive resin, allowing a micromechanical bond with the demineralized substrate.[11,13–15] The self-etch system (SE) does not use prior acid conditioning. The acid monomer present in the adhesive promotes partial dentin demineralization. This procedure partially maintains the smear layer and preserves a greater amount of hydroxyapatite, responsible for protecting the collagen fibrils.[16,17]

The etch-and-rinse strategy (ER) demonstrates better adhesive performance, to the extent that it even leads to cohesive fractures in the enamel, highlighting its high adhesive efficacy.[4,11,15] When compared to the ER mode, SE adhesives exhibited a relatively shorter application time and were less technique-sensitive, thus being considered easy to use.[18] According to the manufacturer, this system proposes greater adhesion and hydrolytic stability, regardless of the conditioning technique and the degree of dentin moisture. It can be used by the ER technique, SE technique or combining both in the selective mode.[4,13,16] Among the most frequently used adhesive brands, Single Bond Universal (3M, St. Paul, MN, USA) has been extensively employed in research studies.[16,17,19] In dentin, the Single Bond Universal adhesive system showed higher bond strength values than the Adper Single Bond adhesive system.[13]

The adhesion of resinous materials to dentin is related to dentin permeability and the depth of adhesive diffusion through the descaling area.[20–22] Therefore, structural alterations of enamel and dentin are generated by higher radiation doses, decreasing the microhardness and bond strength over time.[6,13,23,24] Obliteration of dentinal tubules and fragmentation of collagen fibers can occur during radiotherapy,[2,9,22] negatively interfering with the bond strength.[10,24]

Previous investigation has shown that teeth restored immediately after radiotherapy have lower adhesive resistance than those without radiotherapy.[5,23–25] Others demonstrated that radiotherapy did not affect the bond strength of the adhesives to either enamel or dentin.[2,9]

Therefore, there is no consensus if radiotherapy can affect the organic and mineral phase of dental tissue or hamper the bond strength results. The present study evaluated in vitro the bond strength of the Single Bond Universal adhesive system to dentin submitted to radiotherapy, with two different protocols of the adhesive system (ER and SE). The null hypotheses of the study were:

1) Radiotherapy would exhibit no significant difference in shear bond strength of the adhesive interface, whether restored with etch-and-rinse or self-etch adhesives;

2) There is no difference among groups in the morphology of the adhesive interface.

MATERIALS AND METHODS

Ethical issues and experimental design

After approval by the local Ethics in Research Committee, extracted maxillary canines were obtained and maintained in 0.1% thymol solution. Digital radiographs were taken in ortho and mesioradial angulations. The model followed a randomized block design, with two experimental factors, in two levels (2 x 2): with and without radiotherapy on dentin, treated with two adhesives protocols: etch-and-rinse (ER); self-etch (SE).

Sample selection and preparation

Sixty human maxillary canines, recently extracted, all of them permanent and free of caries, were selected from the local Biobank. The specimens were stored in 0.1% thymol solution for fewer than 30 days before being used, at 9°C and rinsed in running water for 24 h to eliminate the thymol residues.

Afterwards, teeth were examined with a stereomicroscope (Leica Microsystems, Wetzlar, Germany) under x20 magnification. The specimens were submitted to a total dose of 60 Gy (2 Gy per day for 5 consecutive days for 6 weeks). X-rays were emitted with 200kVp and 25mA energy, through a standard 0.3mm copper filter. The X-rays generated in these conditions have a minimum and maximum spectrum of energy emitted from 95 to 200 kV, respectively, and half the value of the beam with 0.62 mm of copper.

The decay of this dose of X-rays in the tissues is approximately 10% at a depth of 0.5 cm. The containers containing the specimens were equidistant from the center of the X-ray beam to ensure uniform distribution of the radiation dose (approximately 2.85Gy/m). During and after irradiation, the specimens were stored in artificial saliva at 37°C.[26]

Following, the specimens of bond strength test were cut using a precision cutting machine into dimensions of 5 x 5 x 3 mm, then they were included in bakelite resin (Arotec, Cotia, SP, Brazil) with an automatic pressure impregnation system (Arotec Pre30; Arotec, Cotia, SP, Brazil) and the intracoronary dentinal surface was facing upwards. After the resin setting time, the dentin surfaces were flattened under irrigation, with sand-papers (Norton; Lorena, SP, Brazil). Then, the surfaces were polished with 1200 grit sandpaper, performing 600 cycles to standardize the smear layer (Figure 1).

The samples were allocated by randomly drawing the groups. In the ER subgroup, dentin was treated with a water-based gel containing phosphoric acid at 37% (Condac, FGM, Brazil) (10 s) followed by air/water spray for 30 s. Two consecutive layers of the Single Bond Universal adhesive (3M, St Paul, MN, USA) were applied (15 s) using a microbrush. Then, an air jet (5 s) was employed to facilitate solvent evaporation.

The adhesive was sub-sequently light-cured using a light source with a power of 1000 mW/cm² and a wavelength of 395 – 480 nm for 10 s (VALO Grand, Ultradent, South Jordan, UT, USA), positioned 1 cm away from the specimen. In the SE subgroup, the adhesive was applied as a single layer (5 s), followed by an air jet (5 s) as well, and light-cured in the same manner as the ER group.

After, a 4-mm thick bisected Teflon matrix, with 3 x 3 x 4 mm cavity was fixed with wax (Lysanda, São Paulo, SP, Brazil). The matrix was positioned under the specimen, in the dentin area, and was filled with composite resin Z350 (3M, St. Paul, MN, USA). After removing the matrix, the resin cube formed under the dentin was polymerized (20 s). The specimens were stored in distilled water at 37 °C for 24 hours.

Debonding SBS test

The resin specimens were positioned in an Instron 2519-106 universal test machine (Instron Corporation, Canton-Massachusetts, USA) with 2 kN load. The chisel used during shear bond strength testing was the wedge-shaped metallic piece (model OD14b).

This device was positioned as closely and parallelly as possible to the adhesive interface. The metallic base ensures a 45° tilt of the specimen to mimic occlusal forces. Force application was executed using a stainless steel rectangular tip at a constant speed of 0.5 mm/min until the restoration fractured. Failure patterns were examined utilizing a 40x stereomicroscope (Leica Microsystems, Wetzlar, Germany) and categorized as follows: adhesive failures, arising at the interface between adhesive and dentin; cohesive to the material, emerging within the resin; cohesive to the substrate, manifesting in dentin; and mixed failures, representing a blend of adhesive and cohesive failure types.

Analysis of the adhesive interface by SEM

Five specimens from each group were sectioned in halves. One hemi section was discarded and the other was ground with 600 and 1.200 grit sandpapers (Norton; Lorena, SP, Brazil) for 30s and with alumina 0.3 µm and 0.05 µm for 5 min. The new specimens were stored in a 2.5% glutaraldehyde solution, with

0.1 mol/ l sodium cacodylate solution (pH = 7.4) (Dermus, Florianópolis, SC, Brazil) at 4°C, for 12 h.
Subsequently, dehydration occurred in ascending alcohol (25°, 50°, 75°, 95° GL) (20 min) of immersion, plus immersion in absolute alcohol (100° GL) (1h). After this period, the hemi sections remained immersed in hexamethyldisilazane solution (HMDS; Ted Pella, Redding, CA, USA) for 10 min.

To clean the adhesive interface, the sections were treated with 35% phosphoric acid (Ultradent Products; South Jordan, UT, USA) for 20 s, followed by a 20-second rinse with a water jet. Subsequently, ultrasonic cleaning was performed for 10 min. Then, the sequence of preparation was performed as described for the analysis of the dentin surface by SEM, using an EvoMa10 scanning electron microscope (Carl Zeiss, Oberkochen, Germany), operating at 20 Kv, using the SmartSemV0 5.04.02.11 software.

Statistical analysis

The SBS data (MPa) were blindly subjected to statistical analysis using SPSS 25 (SPSS Inc, Chicago, IL, USA) using a preset alpha of 0.05. First, the Shapiro-Wilk test to verify normality and Levene test to check the sample homogeneity were conducted.

Then, the data were analyzed using a two-way ANOVA. Failure modes after the debonding test were calculated in percentage. The SEM images were examined by two examiners (inter-examiner kappa = 0.91).

RESULTS

Shear bond strength (SBS) test

Data analysis showed that the dentin specimens with radiotherapy had lower SBS than the specimens without radiotherapy (p<0.0001). The ER mode had lower SBS values compared to the SE mode (p<0.001) (Table 1). No significant difference was observed in the interaction of factors (radiation x adhesive protocol) (p=0.126).

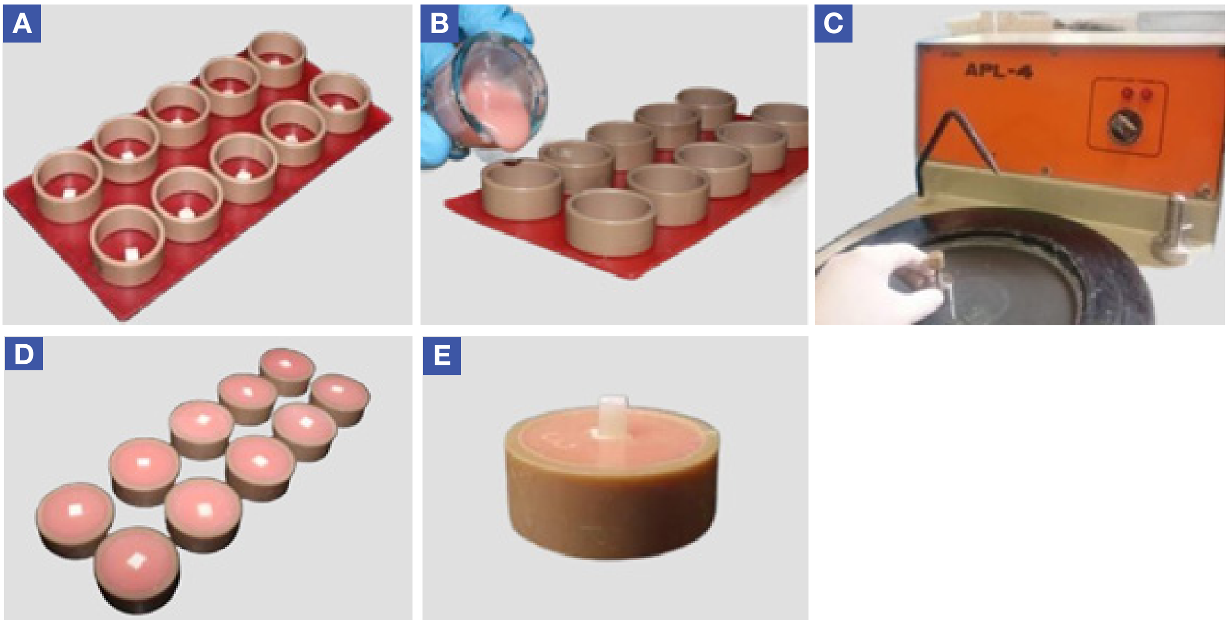
Table 1. Mean (MPa) and standard deviations of the bond strength values of resin to dentin submitted or not to radiotherapy combined with the two bonding strategies of the universal adhesive system (etch-and-rinse and self-etch).

Etch-and-rinse	Adhesive mode	Self-etch adhesive mode
with radiotherapy	10.15 ± 2.59^{Aa}	13.44 ± 1.08^{Ab}
without radiotherapy	14.09 ± 2.97^{Ba}	19.79 ± 2.77^{Bb}

Different capital letters indicate a significant difference in the same column (p<0.05).
Different lower-case letters indicate a significant difference on the same line (p<0.05)

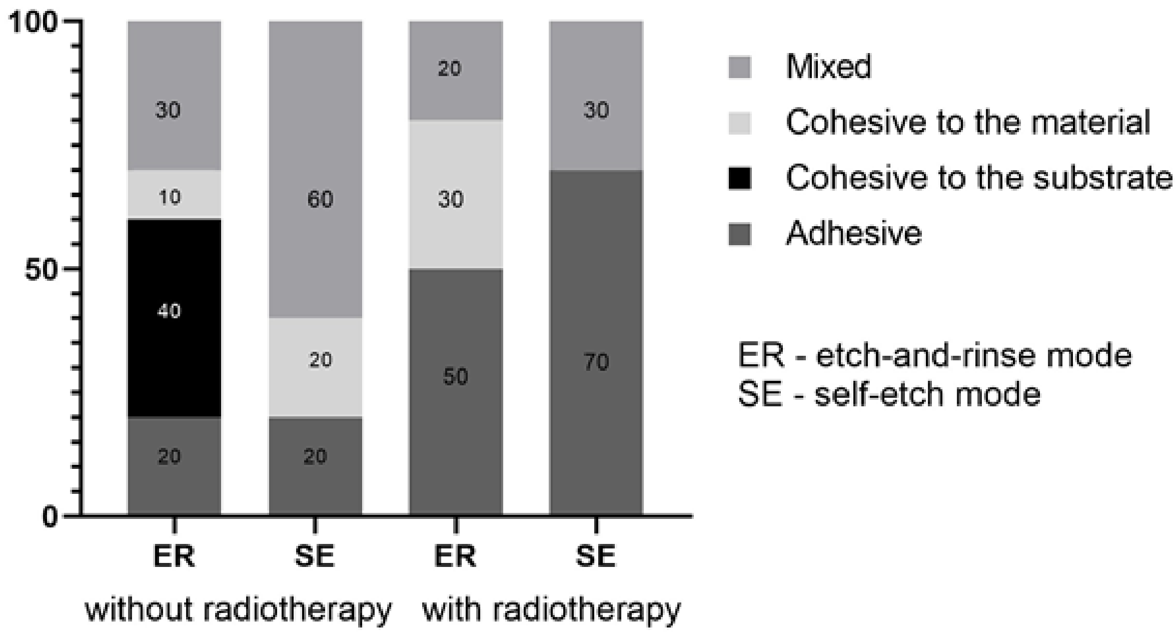
 ISSN Print 0719-2460 - ISSN Online 0719-2479.

Figure 1. Preparation of specimens for the SBS test.



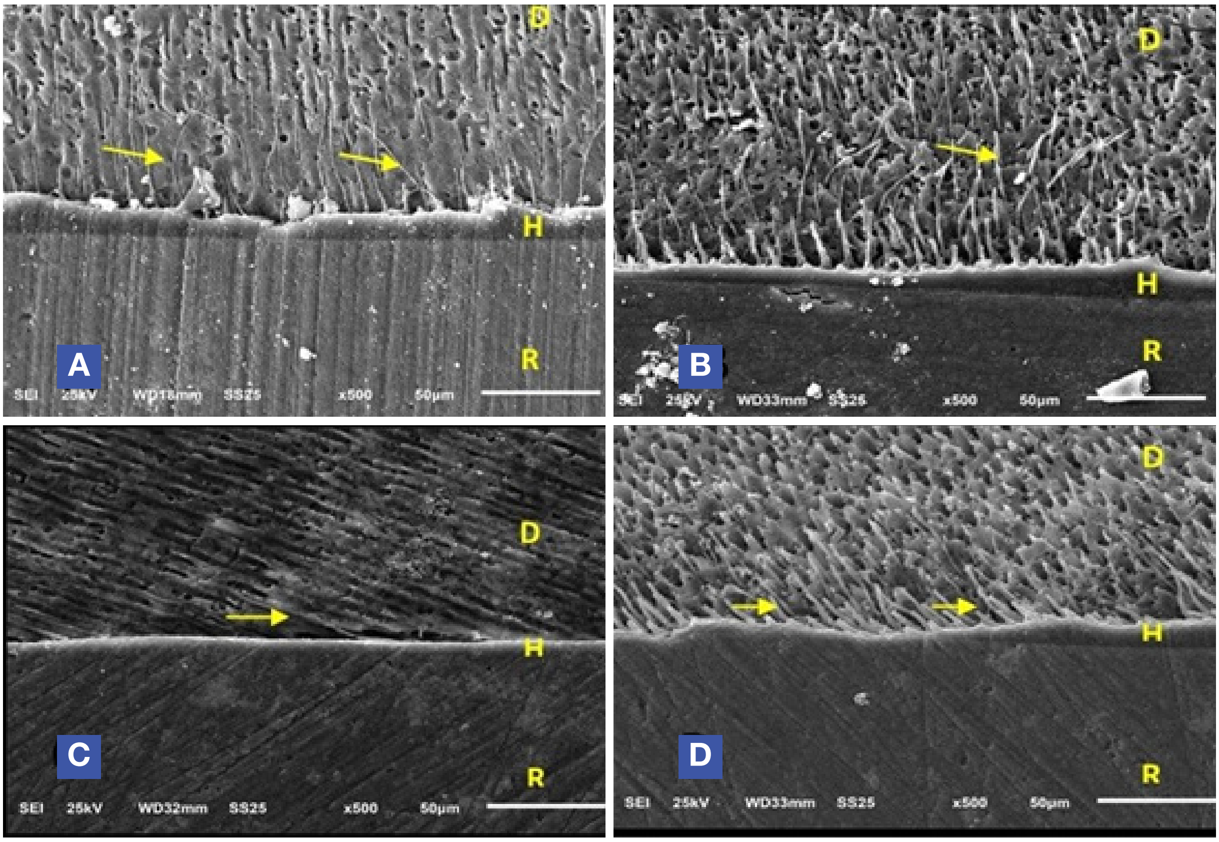
A: Fixing specimens and PVC rings in wax. **B:** Embedding specimens with resin. **C:** Polishing specimens using a polishing machine. **D:** Polished specimens. **E:** Restored specimens.

Figure 2. Failure pattern of dentin/resin (%) after the SBS test on each experimental group (n=10).



J Oral Res. 2023; 12(1): 203-216. https://doi.org/10.17126/joralres.2023.018

Figure 3. Failure pattern of dentin/resin (%) after the SBS test on each experimental group (n=10).



A: Dentin/resin interface without radiotherapy and SE adhesive protocol. **B:** Dentin/resin interface without radiotherapy and ER adhesive protocol. **C:** Dentin/resin interface with radiotherapy and SE adhesive protocol. **D:** Dentin/resin interface with radiotherapy and ER adhesive protocol.
The arrows indicate resin tags. **D:** Dentin. **H:** Hybrid layer. **R:** Composite resin. (x500 magnification).

Failure modes

Adhesive failure was predominant in teeth submitted to radiotherapy, regardless of the adhesive protocol. In teeth without radiotherapy, the prevalence was cohesive to the substrate (ER protocol) and mixed (SE protocol) (Figure 2).

Interface morphology by SEM

The interface of composite resin and dental tissue of teeth without radiotherapy (control) had similar morphological characteristics, regardless of the adhesive protocol (ER or SE). These groups had a hybrid layer and long and thin resin tags inside dentinal tubules (Figures 3A and 3B).

A discrete irregular hybrid layer was observed in the dentin/resin interface of teeth submitted to radiotherapy. In the SE mode with radiotherapy, few tags were presented. In ER mode with radiotherapy, more regular resin tags (Figure 3C) and a demineralized intertubular and peritubular dentin pattern were verified (Figure 3D).

 ISSN Print 0719-2460 - ISSN Online 0719-2479.

J Oral Res. 2023; 12(1): 203-216. https://doi.org/10.17126/joralres.2023.018

DISCUSSION

Treatment for patients with head and neck cancer requires a multidisciplinary view.[6,27] The radiotherapy is the treatment choice for these patients since increased the survival rates.[5,11] Unfortunately, radiotherapy has a semi-selective character, not preserving the surrounding tissues.[3,4,24]

It can lead to certain side effects, such as radiation-induced caries, which begin with tooth discoloration in black or brown shades and the presence of cracks in the enamel. These issues subsequently progress to enamel delamination and can result in significant dental damage, which can lead patients to seek aesthetic treatments.[4,5,8,25,28]

However, some *in vitro* studies have reported difficulties in performing restorative treatments due to compromised bond strength of the resin.[4,23,25,29,30] Therefore, it is crucial to improve the adhesive protocols to prevent potential bond failures in dental treatments.

In this study, we chose to evaluate the Single Bond Universal adhesive, with two adhesion strategies: ER and SE. In the ER system, dentin is conditioned with 35 or 37% phosphoric acid to partially remove the smear layer.[14,31] The selection of the Single Bond Universal adhesive system for this research is grounded in its role as a reliable benchmark, showcasing established adhesive durability with the dental substrate.[20,32] This particular adhesive contains ethanol and water as solvents. Ethanol efficiently permeates the dentin collagen matrix, consequently enhancing the adhesive's bond strength.[33]

Adhesive systems containing hydrophilic primers dissolved in acetone produces higher bond strengths, a protocol referred to as the 'wet bonding technique'.[17] However, the adhesive procedure remains a clinical protocol that is difficult to standardize. Despite this, two-steps SE adhesives demonstrate satisfactory results and are still the standard for dental adhesion in routine clinical practice.[17,19] Our results demonstrate that the shear bond strength of resin to dentin is favored when using SE adhesive protocol, regardless of the radiotherapy pretreatment.

Arid *et al.*,[30] also discovered higher strength values in irradiated teeth for the self-etch adhesive used in their study, thereby reinforcing our findings. On the other hand, Muñoz *et al.*,[13] reported contrasting results, where the etch-and-rinse adhesive system exhibited higher and long-lasting bond strength values in comparison to the self-etch adhesive when subjected to elevated radiation doses.

This disparity from our outcomes can be attributed to the distinct methodologies and radiation doses employed. SE adhesive systems provide greater adhesion capacity to dentin than adhesives that use acid etching previously.[21] These systems do not use acid on dentin, resulting in partial demineralization of the tissue and preserving a greater amount of hydroxyapatite, responsible for the protection of collagen fibrils.[15,19,22]

SE adhesives use hydrophilic acids as monomers that simultaneously enter and decalcify the dental tissue avoiding the partial penetration of the adhesive through the demineralized canaliculus.[19,21] Besides, they increase dentin permeability due to intrinsic acidity, enabling the penetration of resin monomers through tissue microporosities.[19,20]

The analysis of the failure pattern after the debonding test showed a higher percentage of adhesive failures, emphasizing the fragility of the bonding interface. Naves *et al.*,[33] verified, through the microtensile test, that mixed fractures occurred in 85% of the resin restorations on molars previously submitted to radiotherapy.

Galetti *et al.*,[26] observed a predominance of mixed fractures, and that radiotherapy did not affect the dentin bond strength of the adhesive materials. Teeth submitted to head and neck radiotherapy received high doses of radiation, resulting in morphological changes in the teeth.[30] This makes the oral environment more cariogenic and hostile, due to patients' hyposalivation and diet.[27]

Kielbassa *et al.*,[34] observed *in vitro* and *in situ* direct radiation damage of enamel. However, the authors demonstrated that dental hard tissue exposed to radiotherapy is equally susceptible to caries as teeth that have not undergone radiotherapy. It is important to consider that the authors used different doses of radiation. Our methodology simulated the radiation routinely used in the treatment of cancer patients, which showed that the dentin microhardness decreased after 50 Gy compared to 40 Gy and after 60 Gy compared to 50 Gy.[4] Fractional doses of 2 Gy were performed for 5 consecutive days, with 30 cycles, for 6 weeks, making a total of 60 Gy.[1,9,24]

To simulate the oral environment, artificial saliva was used to store teeth,[26] but during radiotherapy, the teeth were immersed in distilled water, since artificial saliva can interfere with radiation due to the viscosity and ions concentration.[2,4,25,27] During irradiation, the excessive water on the dentin decreases vascularization, obliterate tubules, reduces the odontoblast metabolism, and degrades the collagen fibers.[4,8] Clinically, degraded collagen interferes with the hybrid layer,[15] decrease dentin elasticity,[35] and consequently, increase fracture rates.[9,24,25,29]

Photomicrographs of the adhesive interface of teeth with radiotherapy had an irregular hybrid layer and shorter tags than in normal teeth, thus the second null hypothesis was rejected. These teeth have dentin tubules obliterated,[2,4,25] which probably hindered the formation of longer tags, similar to those observed in teeth without radiotherapy. The internal walls of the tubules are coated with mineralized dentin. Intertubular dentin is less calcified and has a higher organic matrix.[24] A previous study also found that radiotherapy caused changes in the dental substrate, with degradation and instability in dentin type IV collagen.[15] Figure 2C is darker than the others due to a microscope device calibration issue.

Overall, the organic matrix of dentin is altered after radiotherapy,[36] reducing the bond strength of restorations.[30] Finally, due to the scarcity of scientific articles on the subject, this study may support the clinical treatment

 ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

J Oral Res. 2023; 12(1): 203-216. https://doi.org/10.17126/joralres.2023.018

of patients undergoing radiotherapy in the head and neck region. The SE adhesive protocol of the universal adhesive system is a promising alternative to restore the tooth after radiotherapy treatment. Furthermore, conducting an analysis after a period of time would be interesting for future research. Moreover, exploring different adhesive brands could enhance the study's validity.

CONCLUSION

In conclusion, the self-etch adhesive protocol of the universal adhesive system favors the shear bond strength of resin to dentin in teeth submitted to radiotherapy compared to the etch-and-rinse mode. Teeth undergoing radiotherapy have an irregular hybrid layer and shorter tags than without radiotherapy.

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

Study received approval from the Intuitional Ethics in Research Committee

FUNDING

Coordination for the Improvement of Higher Education Personnel (CAPES) – DS/CAPES.

AUTHORS' CONTRIBUTIONS

de Goes Paiola F: Investigation, methodology, funding acquisition, visualization, writing, original draft.
Souza-Gabriel AE: Formal analysis, visualization; writing – original draft
Leite Paschoini V: Writing, review and editing.
Mussolino Queiroz A: Conceptualization, supervision.
Miranda Cruz-Filho A: Conceptualization, visualization, writing, original draft, supervision, project administration.

ACKNOWLEDGEMENTS

The authors are thankful to the financial support of Coordination for the Improvement of Higher Education Personnel (CAPES) – DS/CAPES.

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PUBLISHER'S NOTE

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This manuscript was evaluated by the editors of the journal and reviewed by at least two peers in a double-blind process.

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ISSN Print 0719-2460 - ISSN Online 0719-2479.
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 ISSN Print 0719-2460 - **ISSN Online** 0719-2479.